CLINICO-PATHOLOGICAL AND MOLECULAR PROGNOSTIC FACTORS IN RESECTED PANCREATIC CANCER

MD THESIS

RICHARD SMITH

SUPERVISORS:

PAULA GHANEH

CATRIN TUDUR-SMITH

DECLARATION

I hereby declare that this research work was carried out in the Division of Surgery and Oncology, University of Liverpool, United Kingdom from October 2006 to February 2009. The content of this thesis represents original work and has not been presented, either wholly or in part for any other degree or qualification.

Richard Smith

06/01/2012

ACKNOWLEDGEMENTS

I would like to thank the following people for their help, support and guidance during the period of my research at the Division of Surgery and Oncology:

Miss Paula Ghaneh

Dr. Catrin-Tudor Smith

Dr. Fiona Campbell

Prof. John Neoptolemos

PUBLICATIONS FROM CONTENT OF THIS THESIS

Preoperative resolution of jaundice following biliary stenting predicts more favourable early survival in resected pancreatic ductal adenocarcinoma. Smith RA, Dajani K, Dodd S, Whelan P, Raraty M, Sutton R, Campbell F, Neoptolemos JP, Ghaneh P. *Ann Surg Oncol* 2008; 15: 3138-46.

Preoperative CA19-9 levels and lymph node ratio are independent predictors of survival in patients with resected pancreatic ductal adenocarcinoma. Smith RA, Bosonnet L, Ghaneh P, Raraty M, Sutton R, Campbell F, Neoptolemos JP. *Dig Surg* 2008; 25: 226-32.

The platelet-lymphocyte ratio improves the predictive value of serum CA19-9 levels in determining patient selection for staging laparoscopy in suspected periampullary cancer. Smith RA, Bosonnet L, Ghaneh P, Sutton R, Evans J, Healey P, Garvey C, Hughes M, Raraty M, Campbell F, Neoptolemos JP. *Surgery* 2008; 143: 658-66.

Preoperative platelet-lymphocyte ratio is an independent significant prognostic marker in resected pancreatic ductal adenocarcinoma. Smith RA, Bosonnet L, Raraty M, Sutton R, Neoptolemos JP, Campbell F, Ghaneh P. *Am J Surg* 2009; 197: 466-72.

Classification of R1 resections for pancreatic cancer: the prognostic relevance of tumour involvement within 1 mm of a resection margin. Campbell F, Smith RA, Whelan P, Sutton R, Raraty M, Neoptolemos JP, Ghaneh P. *Histopathology* 2009; 55: 277-83.

Meta-analysis of immunohistochemical prognostic markers in resected pancreatic cancer. Smith RA, Tang J, Tudur-Smith C, Neoptolemos JP, Ghaneh P. *BJC* 2011; 144:1440-1451.

ABSTRACT

Backgound - This thesis encompasses two main areas of study relating to factors predicting survival following resection for pancreatic cancer. The first section includes a detailed analysis of various clinico-pathological prognostic factors in a 10-year cohort of patients undergoing pancreatic resection at a single regional tertiary referral centre (1). The second section comprises a systematic review and meta-analysis of published literature investigating several key immunohistochemical prognostic factors in resected pancreatic cancer (2).

Methods - (1) Retrospective clinical and histopathological data were collected for 166 patients undergoing pancreatoduodenectomy for pancreatic adenocarcinoma and entered on to a database. The histopathology slides were retrieved for all resection margin negative (R0) cases and re-assessed to conduct an analysis of the prognostic relevance of 'equivocal' margin involvement (ie. microscopic tumour involvement within 1mm of one or more margins without directly breaching the margin itself). Survival analyses were undertaken to identify the most important prognostic variables with a view to generating a combined prognostic index. (2) p53, smad4, p16, bcl-2, bax, VEGF and EGFR were identified as the most widely investigated and biologically important molecular prognostic factors in resected pancreatic cancer. MEDLINE, EMBASE and ISI Web of Science were used to search for relevant literature and a random effects inverse variance approach was used to analyse the pooled data.

Results - (1) Tumour size, differentiation and lymph node ratio were found to be significant histopathological prognostic factors for overall survival on multivariate analysis. Sub-group analysis of resection margin status indicated no significant survival difference between 'equivocal' and definitive R1 cases. The preoperative

platelet-lymphocyte ratio was identified as a novel prognostic factor and was found to exhibit strong relationships with both invasive tumour characteristics and likelihood of postoperative patient selection for adjuvant therapy. Preoperative serum albumin and CA19-9 were also significant independent prognostic factors on multivariate analysis. A combined score was found to provide superior prognostic information to any individual preoperative or histopathological factor. (2) VEGF (11 studies, n=767), bcl-2 (5 studies, n=314), bax (5 studies, n=274) and p16 (3 studies, n=229) emerged as significant immunohistochemical prognostic markers from the pooled data of meta-analysed studies while p53 (17 studies, n=925), smad4 (5 studies, n=540) and EGFR (4 studies, n=250) returned non-significant results. There was evidence of significant heterogeneity in four of the seven study groups.

Conlusions - The findings from this study provide the first clinical evidence to support use of the '1mm rule' in defining resection margin status for pancreatoduodenectomy reporting. This issue was found to have a significant impact on the overall number of cases classified as R1. The preoperative platelet-lymphocyte ratio is a newly described prognostic marker in resected pancreatic cancer and when analysed alongside serum CA19-9 and albumin levels, comparable prognostic information can be derived from these routine preoperative haematological and biochemical parameters when compared with standard histopathological tumour characteristics. The meta-analysis of immunohistochemical prognostic studies indicates that VEGF represents the most potentially informative prognostic factor of the seven most widely investigated molecular markers identified and should be considered as a comparative marker in future prognostic studies utilising microarrayed tissue from adjuvant therapy trials for resected pancreatic cancer.

CONTENTS **Page**

1. INTRODUCTION

1.1.1. *EPIDEMIOLOGY*

Over 232,000 new cases of exocrine pancreatic cancer are estimated to occur worldwide each year (International Agency for Research on Cancer 2002). Pancreatic cancer is the tenth most common malignancy in the United Kingdom, accounting for 3% of all new cases of cancer each year with 7632 cases diagnosed in 2005 (http://info.cancerresearchuk.org/cancerstats). The aggressive nature of the disease is underlined by the fact that pancreatic cancer represents the sixth most common cause of UK cancer mortality with 7250 deaths per year (Office for National Statistics 2004) - ie. a mortality to incidence ratio approximating 1.0.

Having peaked in the 1970s and 1980s, the UK age-standardised incidence of pancreatic cancer is believed to have fallen slightly over the past 20 years with a consequent similar pattern seen for age-standardised mortality rates (Fitzsimmons et al. 2007; Wood et al. 2006).

Gender

Men and women in the UK have a comparable lifetime risk for developing pancreatic cancer of approximately 1.0%. However, males have a greater age-standardised incidence rate for pancreatic cancer when compared to females (10.1 per 100,000 and 7.7 per 100,000 respectively [Office for National Statistics 2007]). The male-to female ratio of age-standardised incidence rates has gradually decreased over recent years in England and Wales (Wood et al. 2006). The pattern of differences in incidence rates between males and females is likely to be attributable to changing patterns of lifestyle factors due to the association between smoking and pancreatic cancer risk.

Age

Pancreatic cancer incidence rises sharply in patients over the age of 60 years with 85% of cases occurring in patients within this demographic group. 6.9 cases per 100,000 are seen in patients aged 45-49 years compared with 50.1 cases per 100,000 in patients aged 75-79 years (Cancer Research UK 2004).

Socioeconomic status

There is no evidence to suggest that the incidence of pancreatic cancer is higher for UK populations in lower socioeconomic groups when compared with more affluent groups (Wood et al. 2006; Dutta Roy et al. 2005). Only marginal differences in pancreatic cancer incidence between socioeconomic groups have been reported in countries elsewhere in Europe (Ji et al 2006; Weiderpass et al. 2006). These findings are likely to relate to the influence of potential differences in lifestyle and occupational risk factors for pancreatic cancer between different socioeconomic strata. Occupational factors reported to be associated with an increased risk of developing pancreatic cancer include industries associated with printing and paper manufacture, along with petroleum and chemical industries, particularly those involving formaldehyde exposure (Kernan et al. 1999).

Ethnicity

Previous epidemiological data from North America have suggested that the incidence of pancreatic cancer is more common in black populations when compared with caucasian populations (Ries et al. 2002). This finding contrasts with other epidemiological data which have demonstrated that the age-standardised mortality rates for pancreatic cancer (used as a surrogate marker of incidence) are significantly

less in African countries when compared with many developed countries (International Agency for Research on Cancer 2002). This observation has been explained on the basis of lifestyle differences between the different ethnic groups (Silverman et al. 2003). Although Japan and Korea are among the countries with the highest age-standardised mortality rates for pancreatic cancer in the world, the corresponding mortality rates for China, Indonesia and Malaysia are all significantly less (International Agency for Research on Cancer 2002). These differences are similarly believed to be attributed to dietary and lifestyle factors rather than genetically determined risk.

1.1.2. *AETIOLOGY*

Smoking

Cigarette smoking is believed to be the most significant environmental determinant of pancreatic cancer risk. Smokers have been demonstrated to have a two-fold increased risk of developing pancreatic cancer when compared to non-smokers with evidence for a dose-dependent relationship between smoking and cumulative risk (Silverman et al. 1994; Lin et al. 2002). It has previously been proposed that exposure of the pancreatic ductal epithelium to cigarette-derived carcinogens excreted in bile results in the trigger for carcinogenesis (Wynder et al. 1973). In vitro and animal studies have shown that exposure of one of the key carcinogenic tobacco-specific nitrosamines (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone - NNK) to pancreatic epithelial cells results in activation of intra-cellular proliferative pathways (Askari et al. 2005; Al-Wadei et al 2009). NNK has been shown to exhibit significant biliary excretion in rats (Schulze et al. 1992) and has been identified in pancreatic juice from smokers (Prokopczyk et al. 2002). Polymorphisms of genes involved in carcinogen-metabolising protective mechanisms may also be a significant determinant of pancreatic cancer risk in individual smokers (Bartsch et al. 1998; Duell et al. 2002).

Alcohol

Previous studies have suggested that long-standing heavy alcohol consumption may be a contributory risk factor in the development of pancreatic cancer (Go et al. 2005; Ye et al. 2002). However, the extent to which this effect is exerted as a result of alcohol-induced chronic pancreatitis or separate non-pancreatitis related pathways is unknown (Whitcomb et al. 2002; Jelski et al 2007). Additional confounding factors include the propensity for alcoholics to develop type II diabetes mellitus (which may

also represent an additional risk factor for pancreatic cancer) along with the fact that heavy alcohol consumption is invariably associated with cigarette smoking and adverse dietary patterns. In a recent US prospective study including over a million participants, excessive consumption of spirits was associated with a significantly greater pancreatic cancer risk when compared with excessive beer or wine consumption (Gapstur et al. 2011).

Nutrition

As with other malignancies such as colorectal cancer, dietary intake of various carcinogens commonly produced as result of food preparation (eg. polycyclic aromatic hydrocarbons (PAH), heterocyclic amines, etc) is believed to play a role in predisposing to pancreatic carcinogenesis (Silverman et al. 1998; Anderson et al 2002). Conversely, a high dietary intake of antioxidants may provide a protective mechanism against genotoxic free-radical production and DNA adduct formation associated with various carcinogens, whether inhaled or ingested (Woutersen et al. 1999). The extent to which dietary risk for pancreatic cancer is related to, or independent of, smoking and alcohol intake is unknown. However, recent evidence has suggested that a high dietary intake of carcinogens derived from cooked meats increases the risk of pancreatic cancer synergistically alongside cigarette smoking (Li et al. 2007).

Chemopreventive agents

Given the poor survival rates associated with a diagnosis of pancreatic malignancy, much interest has focused on whether potential chemopreventive agents might be able to reduce the risk of developing pancreatic cancer. The proposed anti-cancer effects of non-steroidal anti-inflammatory drugs (NSAIDs) have been most widely investigated in the context of pancreatic cancer prevention and *in vitro* evidence exists to suggest that COX-2 inhibition might influence the progression of carcinogenesis and metastatic potential in pancreatic cancer cells (Wenger et al. 2002; Furukawa et al. 2003). However, the clinical evidence to support a link between NSAID use and reduced pancreatic cancer risk is mixed (Anderson et al. 2002a; Coogan et al. 2000). Similarly, conflicting evidence exists with regard to whether regular aspirin use is associated with a reduced risk of developing pancreatic cancer (Schernhammer et al. 2004; Jacobs et al. 2004).

Evidence has been published suggesting that regular statin use (ie. 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) is associated with a reduced likelihood of pancreatic cancer (Khurana et al. 2007). *In vitro* evidence has also demonstrated an association between statin exposure and reduced metastatic potential in pancreatic cancer cell lines (Kusuma et al. 2002). However, a meta-analysis of population-based studies failed to support a significant association between long-term statin use and reduced pancreatic cancer risk when taken at levels to treat hypercholesterolaemia (Bonovas et al. 2008).

Recent pre-clinical evidence has emerged with regards to the potential anti-cancer effects of beta-blockers in the setting of pancreatic cancer with studies reporting pro-apoptotic properties of beta-adrenergic antagonists on pancreatic cell lines (Zhang et al. 2011) and chemopreventive effects reported in animal models (Al-Wadei et al. 2009). No clinical trials have been conducted to date but a recent retrospective cohort study suggested adverse survival outcomes associated with beta-blocker use in patients with pancreatic cancer (Shah et al. 2011). This study included only a limited number of patients with pancreatic cancer (n=140) and the presence of concurrent cardiac co-morbidity represents a confounding factor in the interpretation of these results.

Curcumin represents a naturally occurring agent derived from the tumeric plant which has been widely investigated as both a therapeutic and chemopreventive agent in the setting of pancreatic cancer. Curcumin has been shown to inhibit pancreatic cancer cell proliferation *in vitro* (Wang et al. 2006) and has also been shown to sensitize pancreatic cancer cells to the cytotoxic effects of gemcitabine in both pancreatic cancer cell lines (Lev-Ari S et al. 2007) and animal models (Kunnumakkara et al. 2007). Its clinical usefulness is potentially limited by its poor bioavailability but a number of clinical trials utilising curcumin alongside palliative chemotherapy are currently being conducted for locally advanced pancreatic cancer (Stan et al. 2010). Several other naturally occurring agents including beta-carotene, vitamin D, vitamin E, isothiocyanates (found in broccoli and cabbage), capsaicin (found in red chilli peppers), resveratrol (found in grape skins) and genistein (found in soy products) represent potential therapeutic or chemopreventive agents for pancreatic cancer (Stan et al. 2010).

Chronic pancreatitis

Chronic pancreatitis is both an aetiological factor and physiological consequence of pancreatic carcinogenesis. Pancreatic cancer commonly results in ductal obstruction with development of progressive features of secondary chronic pancreatic inflammation and fibrosis with consequent exocrine and endocrine insufficiency. Several studies have described an association between chronic pancreatitis of differing aetiological causes and the development of pancreatic cancer (Whitcomb et al. 2002; Maisonneuve et al. 2002). However, the strongest evidence to support a clear causal relationship between pancreatic inflammation and malignancy has been reported in cases of hereditary pancreatitis (Lowenfels et al. 1997). This condition is inherited in an autosomal dominant manner and results from mutations in the cationic trypsinogen gene PRSS-1 in around 80% of cases (Whitcomb et al. 1996; Howes et al. 2005). It has previously been demonstrated that hereditary pancreatitis is associated with a lifetime risk for developing pancreatic cancer of approximately 40% (Howes et al. 2004) and this patient group represents a significant target population who might potentially benefit from pancreatic cancer screening (Vitone et al. 2005).

Diabetes mellitus

As with chronic pancreatitis, the association between diabetes and pancreatic cancer may reflect a causal or consequential one. A previous meta-analysis of studies investigating the relationship between type II diabetes mellitus and pancreatic cancer demonstrated a combined odds ratio for pancreatic cancer of 1.82 in type II diabetics. However, significant heterogeneity existed between the studies included in this analysis and the effect of other potential confounding factors such as smoking and diabetes was not investigated (Huxley et al. 2005). An increased cancer risk was

identified among patients with a recent diagnosis of type II diabetes when compared with patients in whom a diagnosis of diabetes was made greater than five years previously. An additional meta-analysis has also demonstrated a similar increased risk of pancreatic cancer in patients with early-onset type I diabetes (odds ratio = 2.00). However, this finding was based on a smaller number of studies (Stevens et al. 2007).

Genetic predisposition

Several studies have provided significant evidence for familial clustering of pancreatic cancer, suggesting that a genetic predisposition is the causative aetiology in up to 10% of all cases. A number of hereditary cancer syndromes have been shown to be associated with an increased risk of pancreatic cancer. Familial atypical multiple-mole melanoma (FAMMM) syndrome is associated with development of multiple cutaneous naevi progressing to malignant melanoma and FAMMM kindreds exhibiting p16 mutations have been demonstrated to exhibit a 13-fold increase in the risk of pancreatic cancer (Goldstein et al. 1995). Breast-ovarian cancer syndromes associated with *BRCA1* and *BRCA2* mutations and hereditary non-polyposis colorectal cancer (HNPCC) caused by mutations in mis-match repair genes including *hMSH2* and *hMLH1* represent the two other main familial cancer syndromes most commonly associated with increased pancreatic cancer risk (Phelan et al. 1996; Peltomaki et al. 1997). Pancreatic cancer is also variably shown to be associated with less common inherited cancer syndromes including Peutz-Jehgers syndrome (Giardello et al. 2000) and familial adenomatous polyposis, a condition which has been demonstrated to confer a 4.5 fold increased risk of pancreatic cancer when compared with the general population (Giardello et al. 1993).

An additional group of families have been reported in which clustering of pancreatic cancer is seen within first-degree relatives in an autosomal dominantly inherited pattern in the absence of any existing hereditary cancer syndromes. A diagnosis of familial pancreatic cancer (FPC) requires at least two first-degree relatives to have had histologically-confirmed pancreatic adenocarcinoma who cannot be categorised into an existing hereditary cancer syndrome (Hruban et al. 1998). *BRCA2* mutations have been identified in up to 20% of FPC families and the pattern of cancer inheritance in these kindreds has been shown to exhibit features of genetic anticipation with successive generations developing pancreatic cancer at progressively earlier ages (McFaul et al. 2006). Large pancreatic cancer family registries have been established both in Europe and North America to further investigate inherited pancreatic cancer risk in these different groups and to evaluate the potential benefits of secondary screening programmes to facilitate earlier detection of pancreatic malignancy in high-risk families.

801.1.3. *PATHOLOGY*

Neoplasms arising from the pancreas can be broadly classified as either exocrine (ductal or acinar cell) or endocrine according to their tissue of origin. *Table 1* demonstrates the World Health Organisation (WHO) classification for malignant tumours arising from the exocrine pancreas.

Table 1 - WHO classification of malignant pancreatic exocrine tumours (Klöppel et al. 1996)

Description	**SNOMed code**
Ductal adenocarcinoma (infiltrating ductal carcinoma)	M8500
Mucinous noncystic carcinoma (mucinous adenocarcinoma)	M8480
Signet-ring cell carcinoma	M8490
Adenosquamous carcinoma	M8560
Undifferentiated (anaplastic) carcinoma	M8020
Mixed ductal-endocrine carcinoma	M8154
Osteoclast-like giant cell tumour	M8030
Serous cystadenocarcinoma	M8441
Mucinous cystadenocarcinoma	M8470
Intraductal papillary-mucinous carcinoma	M8502
Invasive papillary-mucinous carcinoma	M8503
Acinar cell carcinoma	M8550
Pancreatoblastoma	M8971
Solid-pseudopapillary carcinoma	M8452

Pancreatic ductal adenocarcinoma and variants

Ductal adenocarcinoma accounts for over 90% of all pancreatic malignancies and is taken to be synonymous with the term 'pancreatic cancer'. Approximately 75% of lesions arise in the head of the pancreas and the tumour is frequently associated with intense microscopic evidence of desmoplasia reflecting a stromal host inflammatory reaction to the tumour mass. Features of intrapancreatic perineural invasion are invariably seen which may extend to involve the extrapancreatic nerve plexus. Lymphatic, direct and haematogenous spread are common early events in the natural history of pancreatic ductal adenocarcinoma which frequently precede the development of symptoms resulting in the majority of patients exhibiting locally advanced or metatstatic disease at presentation.

Immunohistochemical study of ductal adenocarcinomas demonstrate that these tumours characteristically express cytokeratins (CK7,8,18,19) indicating their epithelial origin, along with overexpression of the tumour markers CA19-9 and carcinoembryonic antigen (CEA). Mucinous (non-cystic) adenocarcinoma, signet-ring cell carcinoma and mixed ductal-endocrine carcinomas reflect histological variants of pancreatic ductal adenocarcinoma.

Intraductal papillary mucinous neoplasms (IPMNs)

IPMNs account for over 20% of all cystic neoplasms arising from the pancreas and represent a group of tumours arising from the main pancreatic duct or side branches which exhibit a mucin-producing columnar or papillary epithelium with macroscopic cystic appearances. This description encompasses a morphologically diverse array of different lesions which have been variably referred to over recent decades (eg.

mucinous ductal ectasia). However, a standardised definition was published in 1996 to classify the description of this category of cystic pancreatic lesions (Kloppel et al. 1996). IPMNs are known to have malignant potential and previous series have demonstrated that approximately 25-40% of resected IPMNs exhibit invasive histological features. Malignant transformation results in either a ductal (tubular) or colloidal histological sub-type, the latter of which is associated with more favourable survival outcomes (Adsay et al. 2003). IPMNs arising from the main pancreatic duct have a greater likelihood of malignant transformation when compared with those arising from side-branches and should be considered for surgical resection over surveillance. Other unfavourable characteristics for malignancy include IPMNs greater than 3cm in size, generalised pancreatic duct dilatation and the presence of concurrent symptoms (Tanaka et al 2006).

Acinar cell carcinoma

These tumours are uncommon and account for only 3% of all pancreatic malignancies. Acinar cell carcinomas characteristically form macroscopic nodular lesions and exhibit evidence of enzyme production with consequent positive staining for trypsin on immunohistochemistry. Acinar cell carcinomas confer a similarly poor prognosis when compared with ductal adenocarcinoma.

1.1.4. *PanIN PROGRESSION MODEL*

An association between pancreatic cancer and the presence of potential epithelial precursor lesions incorporating a spectrum of morphological abnormalities including metaplasia, hyperplasia and dysplasia was proposed over 50 years ago (Sommers et al. 1954). Since then, subsequent authors have made similar observations (Cubilla et al. 1976) but it was not until later that the term PanIN (pancreatic intraepithelial neoplasia) was coined (Klimstra et al. 1994). A formal classification scheme was subsequently proposed by Hruban et al in 2001 (*Table 2*).

Table 2 - Classification of Pancreatic Intraepithelial Neoplasia.

Description	PanIN category
Flat epithelial lesions composed of tall columnar cells with basally located nuclei and abundant supranuclear mucin.	PanIN-1A
Epithelial lesions that have a papillary, micropapillary or basally pseudostratified architecture, but are otherwise identical to PanIN-1A.	PanIN-1B
Mucinous epithelial lesions that may be flat but are mostly papillary. By definition, these lesions exhibit mild or moderate nuclear atypia which falls short of those seen in PanIN-3.	PanIN-2
Usually papillary or micropapillary lesions with severe cellular atypia / dysplasia. The lesions resemble carcinoma at the cytonuclear level without demonstrable features of invasion.	PanIN-3

Subsequent studies have provided evidence for a step-wise accumulation of genetic abnormalities resulting in transformation of normal pancreatic ductal cells into PanIN-1 with subsequent progression to PanIN-2, PanIN-3 and eventually invasive adenocarcinoma. Mutations of the proto-oncogene K-ras are believed to represent an early event triggering the development of hyperplasia and initiation of the PanIN

pathway. Previous studies have demonstrated a high rate of K-ras mutations in cancer-associated PanINs (Lohr et al. 2005). Inactivation of the tumour suppressor gene p16 is also believed to be an early event during PanIN progression. Loss of nuclear p16 expression has similarly been shown to represent a common finding in cancer-associated PanIN-1 and PanIN-2 lesions (Wilentz et al. 1998). Loss of smad4 expression along with p53 mutation are, in contrast, believed to be relatively late events during the PanIN progression model which may contribute to the transition from PanIN-3 to invasive malignancy (Maitra et al. 2003; Wilentz et al. 2000).

1.1.5. *PANCREATIC CANCER AND PROGNOSIS*

Pancreatic cancer is among the most biologically aggressive of all human malignancies with mortality rates approximating incidence. Less than 15 - 20% of patients typically present with resectable disease and overall survival for all stages of pancreatic cancer is consequently only around 3 to 5 months. Overall 5-year survival rates are similarly dismal (0.5% to 3%). Surgical resection is the only potentially curative intervention with median postoperative overall survival times of 12 to 18 months typically quoted and 5-year survival rates in the region of 10%. However, adjuvant chemotherapy has been shown to confer a significant survival benefit with 5-year survival rates in excess of 20% (Neoptolemos et al. 2004; Oettle et al. 2007).

The identification of factors which are able to predict survival in pancreatic cancer is important for a number of reasons. Primarily, factors which are demonstrated to directly influence survival (eg. molecular markers expressed in tumour material) may provide insight into the underlying disease process and reveal therapeutic targets for research into disease-modifying drugs. The identification of standardised prognostic factors also allows meaningful comparisons of outcome between different studies which may exhibit significant differences in case mix. Furthermore, risk stratification as part of clinical trials is important in identifying whether certain sub-groups of patients are more or less likely to benefit from specific treatment modalities. Finally, being able to provide an accurate and honest appraisal of prognosis is important in patient counselling whether in a pre- or postoperative setting.

1.1.6. *MOLECULAR PROGNOSTIC FACTORS*

Oncogenes

K-ras belongs to the ras family of proto-oncogenes and is located on chromosome 12. Ras proteins bind to GTPase-activating protein (GAP) and regulate signal transduction across the cell membrane, thereby regulating a number of cellular processes including differentiation and proliferation. In pancreatic cancer, the K-ras gene is most commonly affected by point mutations of codon 12 resulting in a number of different subtypes of mutation - aspartic acid (GAT), valine (GTT), arginine (CGT) and cysteine (TGT). The mutant form of the ras protein results in a GTPase domain which is no longer able to be inactivated by GAP, resulting in a constitutively active form of ras which predisposes the cell to uncontrolled proliferation and malignant transformation.

K-ras mutations are observed in around 80% to 90% of pancreatic adenocarcinoma cases (Lemoine et al. 1992; Allison et al. 1998). Most studies investigating the prognostic relevance of K-ras mutations in resected pancreatic cancer fail to demonstrate a significant relationship between the presence of K-ras mutations and survival (Allison et al. 1998). However, it has previously been shown that the subtype of K-ras mutation may be more relevant in determining prognosis (Kawesha et al. 2000; Immervoll et al. 2006).

Tumour suppressor genes

p53 is a tumour suppressor gene located on chromosome 17 which codes for a gene product (the p53 protein) with a central role in inducing growth arrest and apoptosis in cells which sustain DNA damage. In normal cells, p53 is bound to MDM2 which

maintains p53 in its inactive form. DNA damage initiates cell cycle checkpoint proteins to phoshphorylate p53 into its active form which also results in a significant increase in its half-life. The active form of p53 binds to DNA which in turn activates expression of p21. p21 is a downstream target of p53 which inhibits cyclin-dependent kinases, resulting in cell cycle arrest which either leads to subsequent DNA repair or apoptosis. Point mutations of p53 result in a more stable form of the protein which is unable to bind to DNA and activate p21, allowing the cell to escape growth arrest.

50% to 75% of pancreatic cancers exhibit p53 mutations, most commonly in exons 5 to 8 (de Braud et al. 2004). Nuclear accumulation of p53 detected by immunohistochemistry has been widely investigated as a potential prognostic factor in resected pancreatic cancer. However, only a relatively small number of studies report a significant relationship between overexpression of p53 and less favourable survival (Linder et al. 1997; Digiuseppe et al. 1994). p21 expression has not been demonstrated to have any significant prognostic value in pancreatic cancer (Song et al. 1996; Coppola et al. 1998).

p16 (CDKN2A) is a tumour suppressor gene located on chromosome 9 which is also believed to play an important role in pancreatic carcinogenesis. p16 is a cell-cycle checkpoint protein which binds to cyclin-dependent kinases resulting in cell cycle arrest at the G1/S checkpoint. Around 80% of pancreatic cancers have been demonstrated to lack expression of p16 on immunohistochemistry (Kawesha et al. 2000). However, the prognostic value of p16 expression in resected pancreatic cancer is variably reported (Kawesha et al. 2000; Naka et al. 1998).

The smad4 (or DPC4) gene codes for a protein which is involved in the intracellular signalling pathway of transforming growth factor β (TGF-β). The smad4 protein forms heterodimers in the cytoplasm with activated smad proteins 1, 2 and 3 which translocate to the nucleus activating gene transcription. Loss of smad4 expression, therefore, inhibits TGF-β signalling with consequent loss of its inhibitory effect on cell proliferation. Loss of smad4 expression is observed in approximately 50% of resected pancreatic cancers (Tascilar et al. 2001). Contradictory evidence exists with regard to whether loss of smad4 expression in resected pancreatic cancer has an adverse (Tascilar et al. 2001) or beneficial effect (Biankin et al. 2002) on patient survival.

Apoptotic factors

The ability of cancer cells to evade apoptotic pathways is believed to be an important mechanism in the pathogenesis of pancreatic cancer. The bcl-2 family of apoptotic genes codes for a number of proteins (including bcl-2, bcl-x, bax and bak) which exert either a pro-apoptotic or anti-apoptotic effect. These proteins are believed to mediate release of cytochrome C in the cytoplasm which in turn activates the effector caspases-3 and 9 which trigger breakdown of the intracellular cytoskeleton and the subsequent sequence of events resulting in apoptotic cell death. In pancreatic cancer, bcl-2 expression is variably reported to be a predictor of more favourable survival following resection (Dong et al. 2005; Bold et al. 1999) and this survival pattern is similarly reported for bax immunoreactivity (Friess et al. 1998). In contrast, bcl-x expression has been demonstrated to be a predictor of adverse survival in pancreatic cancer (Evans et al. 2001).

Growth factors and receptors

Epidermal growth factor receptor (EGFR) is the cell surface receptor for a family of extracellular ligands which include EGF and TGF-α and is coded for by the c-erbB1 proto-oncogene. Activation of EGFR stimulates intracellular tyrosine kinase phosphorylation with downstream activation of a number of signalling cascades including the MAPK (mitogen-activated protein kinase) and Akt (protein kinase) pathways which promote cell proliferation. Overexpression of EGFR is reported in up to 70% of pancreatic cancers. However, the prognostic value of immunohistochemical EGFR status is variably reported (Smeenk et al. 2007; Bloomston et al. 2006). Studies investigating the prognostic relevance of the EGF ligand expression in resected pancreatic cancer have demonstrated similar results (Dong et al. 1998, Gansauge et al. 1998). Transforming growth factor-β1 has also been investigated as a potential immunohistochemical prognostic marker in pancreatic cancer. However, conflicting evidence exists with regard to whether the presence of positive TGF-β expression correlates with poorer patient survival (Friess et al. 1993) or more favourable patient survival (Coppola et al. 1998; Nio et al. 2005).

Vascular endothelial growth factor (VEGF) represents a family of four signalling proteins (isoforms A, B, C and D) which stimulate angiogenesis, promote chemotaxis of inflammatory cells and increase vascular permeability. These ligands have three transmembrane receptors (VEGF-1, -2 and -3) which promote intracellular tyrosine kinase cascades when activated. Several studies have suggested a significant association between VEGF expression in pancreatic cancer and poorer survival following resection (Kurahara et al. 2004; Knoll et al. 2001; Ikeda et al. 2001).

1.1.7. *HISTOPATHOLOGICAL PROGNOSTIC FACTORS*

Although several single-centre studies have reported the prognostic impact of tumour histology in the setting of resected pancreatic cancer, relatively few published studies incorporate sizeable patient numbers to generate high-powered analyses. Two such studies presenting data from the Johns Hopkins School of Medicine (Pawlik et al. 2007) and the ESPAC-1 trial (Bassi et al. 2005) which have analysed the prognostic value of histopathological tumour characteristics in large series of resected pancreatic cancer patients (n=905 and n=418, respectively) have demonstrated that tumour size, differentiation and nodal involvement represent independent prognostic factors for overall survival following surgery. Both studies failed to demonstrate a significant independent association between resection margin status and survival on multivariate analysis. Although other tumour characteristics are often also reported as potential prognostic factors (eg. perineural invasion, vascular invasion, T stage, etc), these additional factors are often inconsistently correlated with survival on multivariate analysis (eg. Pawlik et al. 2007).

Pawlik et al also demonstrated that the ratio of involved lymph nodes to sampled lymph nodes in resected pancreatic cancer specimens provides superior prognostic information to overall nodal status (ie. positive vs. negative). This finding has also been mirrored in other gastrointestinal malignancies (Berger et al. 2005; Inoue et al. 2002). These findings indicate that tumour size, differentiation and lymph node ratio represent the key histopathological prognostic factors to use when conducting comparative multivariate analyses of potential supplementary prognostic factors.

Two recent studies have indicated that tumour involvement of the resected pancreatic cancer specimen on microscopic histopathological examination is commonly under-reported in the literature (typically 20% to 40%). These two studies (Verbeke et al. 2006; Esposito et al. 2008) demonstrated R1 rates of 85% and 76% respectively when using the Royal College of Pathologists guidelines (Campbell et al. 2002). These guidelines stipulate that tumour involvement within 1 mm of a resection margin should result in a R1 classification. However, the rationale for this criterion was based on studies investigating circumferential margin involvement in colorectal cancer and no evidence exists to suggest that this principle of microscopic margin involvement within 1 mm is directly applicable to pancreatic cancer. The prognostic impact of multifocal resection margin involvement (ie. where >1 involved resection margin is present in a single specimen) and the relevance of individual margin location are also unknown. Few studies have investigated how other histopathological tumour characteristics influence likelihood of margin involvement (Raut et al. 2007).

1.1.8. *PREOPERATIVE PROGNOSTIC FACTORS*

CA19-9

Carbohydrate antigen 19-9 (CA19-9) is a sialyated Lewis blood group antigen expressed in normal pancreatic ductal cells and is also secreted in a mucin-bound form by the biliary and gallbladder mucosa and excreted in bile. Obstructive jaundice will commonly precipitate elevated serum concentrations and around 5% of the population are believed to lack the Lewis antigen glycosyl transferase enzyme required to synthesize CA19-9 (Itzjowitz et al. 1986). A cut-off level of >37 kU/l is generally used as the optimal point at which pancreato-biliary malignancy can be differentiated from benign disease in symptomatic patients (Goonetilleke et al. 2007). Normalisation of CA19-9 levels following resection for pancreatic cancer has been shown to be associated with a significant improvement in subsequent survival (Sperti et al. 1993; Montgomery et al. 1997; Safi et al. 1998). However, relatively few studies have investigated the potential role of preoperative CA19-9 levels in isolation as a prognostic index (Kau et al. 1999; Lundin et al. 1994; Ferrone et al. 2006).

Lymphocyte count

The immune status of patients is increasingly recognised as a key determinant of cancer outcomes. Pancreatic cancer exhibits a number of mechanisms by which the tumour can escape immune surveillance and inhibition of lymphocyte function secondary to release of the inhibitory cytokines interleukin-10 and TGF-β is believed to be one such mechanism (Bellone et al. 1999). Only two previous studies have investigated the potential prognostic value of low preoperative lymphocyte counts in resected pancreatic cancer (Yamaguchi et al. 2000; Fogar et al. 2006). These studies included a limited number of patients (n=14 and n=23, respectively). However, both

suggested a significant association between preoperative lymphocytopaenia and poorer survival.

Platelet count

A similarly small number of studies (n=3) have investigated the relationship between preoperative platelet count and survival in resected pancreatic cancer. These studies are conflicting in that two suggest a relationship between thrombocytosis and adverse survival (Suzuki et al. 2004; Brown et al. 2005) while an additional study indicated the opposite relationship with lower platelet counts exhibiting an association with less favourable survival (Schwarz et al. 2001). However, the latter study did include a mix of both pancreatic and periampullary cancers in the survival analysis.

C-reactive protein (CRP)

Although the association between elevated serum CRP levels and adverse survival has been widely investigated in advanced pancreatic cancer, only a single study (Jamieson et al. 2005) has investigated the potential prognostic value of preoperative CRP levels in resected pancreatic cancer. This study demonstrated a significant relationship between elevated preoperative CRP and poorer survival on univariate, but not multivariate analysis.

1.1.9. SYSTEMATIC REVIEW & META-ANALYSIS OF PROGNOSTIC STUDIES

A systematic review represents a literature-based project which is intended to comprehensively identify and critically review all published and unpublished literature relevant to a specific area of interest. This provides the opportunity to critique recent developments within a field of research, to highlight and rationalise potential contradictory findings between different studies and to identify avenues for future research activity. Systematic reviews play an important role in summarising and disseminating findings from clinically-oriented research with a view to facilitating the process of delivering evidence-based medical practice (Sackett et al. 1997).

Meta-analysis can be defined as 'the statistical analysis of a large collection of results from individual studies for the purposes of integrating the findings' (Glass et al. 1976). Meta-analysis of data from multiple sources (typically randomised controlled trials) provides the opportunity to generate pooled statistical analyses which can provide comprehensive answers to specific clinical questions and help to resolve potential disagreement between individual studies. Meta-analysis can involve collation of raw data from multiple trials or studies allowing detailed re-analysis of outcome measures (ie. an individual patient data meta-analysis). However, this approach is labour intensive and necessitates co-operation from all authors whose literature is included as part of the meta-analysis. In practical terms, this is often prohibitively difficult to carry out (Lyman et al. 2005). More commonly, data is extracted indirectly from published literature to generate pooled results (ie. an aggregate patient data meta-analysis).

The important components of a systematic review / meta-analysis can be summarised as follows:

- Clearly-defined study objectives and inclusion/exclusion criteria.
- Use of a standardised and systematic approach for the identification of relevant literature.
- Tabulation of study characteristics and quality assessment of methodology.
- Data extraction from eligible studies where possible (and appropriate) in order to combine data to generate more powerful statistical analyses.
- To form appropriate conclusions and generate recommendations or best-practice guidelines.

Search strategy

Comprehensive identification of all published medical literature relating to a specific study question necessitates use of web-based search engines. Use of multiple search engines is recommended in order to maximise the number of potentially eligible studies returned. In the context of systematic reviews and meta-analyses targeting primary studies as opposed to randomised trials, MEDLINE, EMBASE and ISI Web of Science represent the most widely utilised search engines covering US, European and International journals. In order to minimise the potential for publication bias, a search for non-published studies is also recommended and there are a number of web-based resources which allow identification of abstracts presented at conferences and meetings which may not have been subsequently published as full papers (eg. ISI Proceedings, American Society of Clinical Oncology (ASCO), etc). These strategies can be supplemented by checking reference lists of relevant studies and existing review articles. Search criteria should be carefully selected to optimise the ability to

identify a manageable volume of pertinent literature without excluding potentially relevant studies. Searches and assessment of study eligibility should ideally be performed by more than one researcher.

Quality assessment

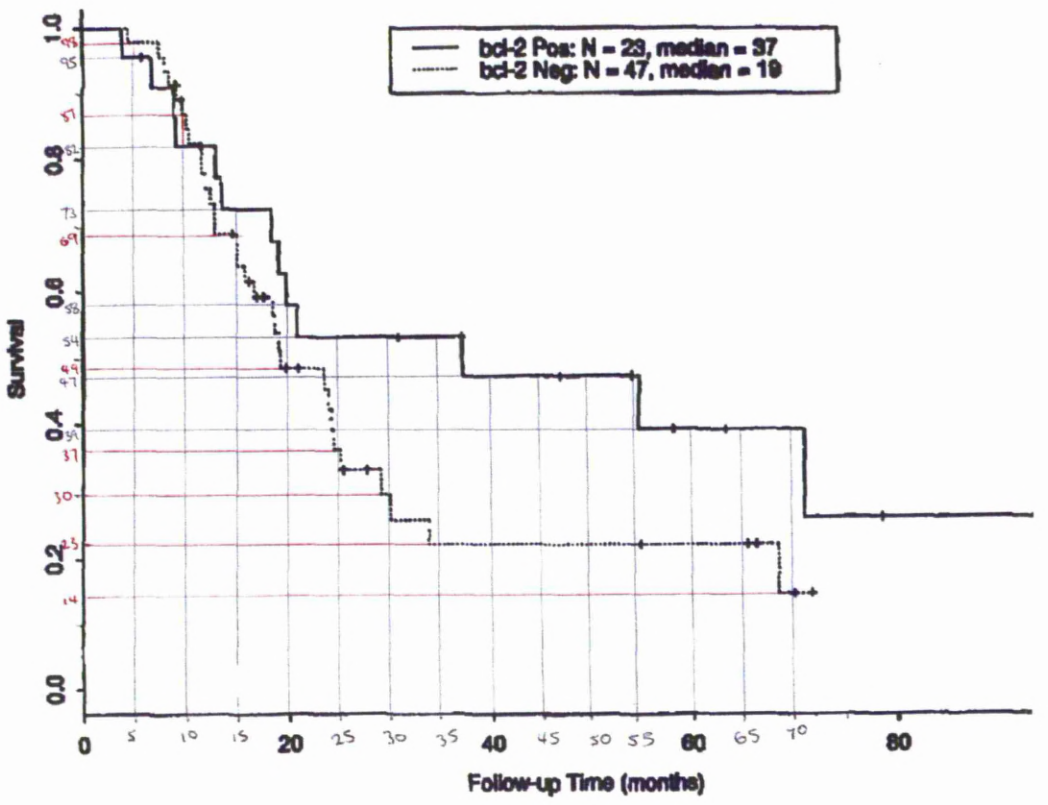
Poorly conducted clinical research yields results which are open to criticism. It is, therefore, desirable to make an assessment of methodological quality of included studies when conducting a meta-analysis to ensure that meaningful conclusions can be drawn. With regard to randomised trials, the CONSORT (Consolidated Standards of Reporting Trials) guidelines represent an internationally agreed framework for high quality trial design and methodology (Moher et al. 2001) and recognised scoring systems for assessing trial quality exist (eg. Jadad et al. 1996). Although there are no such standardised scoring systems for assessing methodological quality of prognostic studies specifically, literature exists which outlines the general prerequisites which should be fulfilled for such studies (Hayden et al. 2006).

Data extraction and analysis

As previously outlined, attempting to obtain raw data from a number of authors for primary studies identified as part of a meta-analysis is invariably impractical. Consequently, it is usually necessary to obtain data from the published results of such studies to conduct an aggregate patient data analysis. For the purposes of prognostic literature, time-to-event data (ie. survival times) are the end-point of interest and are summarised as hazard ratios (HR). This function can be equated to the instantaneous relative risk of death associated with one group when compared with a baseline or control group and can be estimated from regression models (eg. Cox regression)

where the HR represents the inverse natural logarithm of the regression coefficient (e^{β}). Where comparative survival data from two patient groups is the outcome measure of interest, the logHR and variance are required to pool data for meta-analysis. This summary statistic is rarely reported in most prognostic studies. However, there are a number of reported methods (Parmar et al. 1998) for estimating this value indirectly using the summary statistics which are commonly utilised in published literature - eg. the HR and 95% confidence interval or the log rank p-value and the number of events recorded in each study group. Alternatively, the Kaplan-Meier survival curves can be used to record the comparative % survival for set time intervals along each survival curve. Along with the sample size of each group, this information can be used to obtain an estimate for the logHR and variance (*fig.1*).

fig.1 - Example of indirect method of extracting survival data from K-M curves (Bold et al. 1999).



Dedicated software is available to generate the indirect estimates of logHR and variance from the summary statistics outlined above (www.biomedcentral.com /content/supplementary/1745-6215-8-16-S1.xls).

Once logHR and variance have been calculated for individual studies, these values can be pooled in order to generate a weighted average. An inverse variance method is commonly employed for aggregated survival data whereby less weighting in the pooled estimate is apportioned to studies reporting a greater logHR variance. A fixed or random effects approach must be selected based on the assumption of whether the true survival effect of the prognostic parameter of interest is uniform or random across the included studies. Where there is evidence of significant heterogeneity in the direction and/or magnitude of the reported survival effect, a random effects model should be utilised which incorporates between-study variation in the calculation of the overall result (DerSimonian et al. 1986). Assessment of heterogeneity as part of a meta-analysis represents a measure of the consistency or disparity in the true treatment effect across studies. Heterogeneity may be assessed with use of Cochran's chi-squared test or the I^2 statistic (Higgins et al. 2003). In cases where significant heterogeneity is observed across studies included in a meta-analysis, potential reasons for disparity should be investigated and use of a random effects approach is required if studies can be meaningfully grouped for analysis. A random effects approach is also advocated in situations where a limited number of studies are included for meta-analysis.

Systematic review and meta-analysis of prognostic studies present a number of different challenges (Altman et al. 2001). Some of these potential difficulties are common to all systematic reviews and meta-analyses (eg. reliably identifying all published and unpublished literature, incomplete reporting of salient survival data, etc). However, comparing and collating data from different prognostic studies is also associated with several specific problems. These include disparate laboratory methodologies for assessing prognostic factors, variation in patient inclusion/exclusion criteria used, the prevalence of retrospective study design, non-standardised approaches to analysing and reporting survival data along with differing methods for analysis of continuous prognostic variables. Adequate consideration should be given to all these factors prior to attempting meta-analysis of prognostic studies.

1.2. STUDY OBJECTIVES

Clinico-pathological prognostic factors:

- To conduct a comprehensive analysis of histopathological prognostic factors in patients undergoing resection for pancreatic cancer at a high-volume tertiary referral centre over a 10-year period.
- To conduct a detailed analysis of how resection margin involvement influences postoperative survival and whether the criteria for 'equivocal' margin involvement (ie. <1 mm) should be considered synonymous with margin positivity in pancreatic cancer.
- To identify the potential prognostic value of various preoperative biochemical and haematological factors including CA19-9, bilirubin, albumin, CRP, lymphocyte and platelet counts with a view to generating an overall predictive scoring system.
- To investigate the influence of preoperative markers of systemic inflammation on pancreatic and periampullary tumour resectability along with an analysis of any relationships between these indices and histological tumour characteristics. The implications for patient selection for staging laparoscopy were also investigated as part of this analysis.

Systematic review and meta-analysis of molecular prognostic literature:

- To conduct a detailed systematic review of published and unpublished literature assessing the prognostic value of the most widely investigated and biologically relevant molecular prognostic markers in resected pancreatic cancer.
- To conduct a subsequent meta-analysis of the relevant studies in order to estimate the pooled prognostic effect of individual molecular factors analysed as univariate prognostic variables.
- To conduct an analysis of methodological quality for the relevant studies in order to identify potential reasons for the different survival trends observed between individual studies.

2. CLINICO-PATHOLOGICAL PROGNOSTIC FACTORS

2.1. *METHODS*

Details of all patients referred to the Pancreatic Unit in The Royal Liverpool University Hospital since January 1997 have been prospectively recorded and maintained on a Microsoft Access database. All patients undergoing resection for pancreatic and peri-ampullary malignancy between January 1997 and December 2007 were identified from this source. From this list, only cases undergoing pylorus-preserving partial pancreatoduodenectomy or a classical Kausch-Whipple resection were selected for further analysis of histological prognostic factors. This list was cross-checked against records of pancreatoduodenectomy specimens kept in the Pathology department to ensure no missing cases were omitted.

Clinical data collected from the database included patient demographics, details of preoperative biliary stenting, outcome of CT assessment and laparoscopic staging (ie. with regard to assessment of tumour resectability). Preoperative blood results including CA19-9, liver function and full blood count estimations were separately collected from hospital computer records. Overall survival data were collected by identifying the recorded date of death from hospital computer records. For living patients and the small proportion of cases lost to follow-up, the date of last clinic attendance was used to calculate the censored time for survival analyses. All patients gave written consent for clinico-pathological data to be used for research purposes.

All patients with potentially resectable pancreatic tumours and obstructive jaundice presenting during the study period routinely underwent biliary decompression at endoscopic retrograde cholangiopancreatography (ERCP) where possible. Where

patients underwent more than one procedure prior to successful stenting, the date of definitive biliary drainage was used for analysis. Those in whom endoscopic drainage was unsuccessful went on to undergo percutaneous transhepatic cholangiography (PTC) or combined procedures with internal stenting. External drainage was required in a proportion of these cases. A plastic biliary endoprosthesis was routinely used and a covered metallic biliary stent was required in a small proportion. Jaundice was defined as a serum bilirubin concentration of >35 μmol/l. This level was selected as hyperbilirubinaemia is usually only clinically evident as jaundice when serum bilirubin levels exceed this value.

Pylorus-preserving pancreatoduodenectomy (PPPD) was considered the operation of choice for pancreatic head and peri-ampullary tumours for the duration of the study period due to the associated preservation of gastrointestinal function and comparable oncological radicality when compared with a classical approach in previous randomised trials (Seiler et al, 2005; Tran et al, 2004). In cases where the viability of the remaining duodenal margin was uncertain or local tumour infiltration into the pylorus was present, a classical Kausch-Whipple's procedure (ie. with concurrent distal gastrectomy) was performed. Concurrent cholecystectomy was routinely undertaken alongside the pancreatoduodenectomy if the patient still had their gallbladder at the time of surgery.

Details of adjuvant therapy received were collected from the Liverpool Cancer Trials Unit. A search of case report forms was conducted to identify all participants of ESPAC-1 and ESPAC-3 who underwent resection at the Royal Liverpool University Hospital. The small number of patients who received neoadjuvant therapy or off-trial

adjuvant therapy were also identified from departmental records. Patients were considered for adjuvant therapy as part of either ESPAC-1 or ESPAC-3 during the study period primarily on the basis of their postoperative progress and performance status following surgery. Those patients who made a good functional recovery following resection were routinely referred to the regional oncology service and offered the option of trial participation. Randomisation and commencement of treatment was routinely undertaken within 6 to 8 weeks of surgery.

Resectability study

A separate cohort of patients was identified in order to assess the predictive values of the preoperative haematological and biochemical parameters for determining tumour resectability. Details of all referrals between January 1997 and September 2006 with suspected pancreatic/periampullary malignancy were prospectively collected and maintained on a database. Patients undergoing contrast-enhanced computed tomography (CT) were identified to select a group with radiologically resectable disease at presentation. Decision-making regarding resectability was undertaken during weekly Multi-Disciplinary Team meetings. The principal CT criteria used to determine resectability were based on the presence of intra- or extra-abdominal metastatic disease and on vascular encasement or tumour involvement of the superior mesenteric-portal vein over >50% circumference and/or >2 cm length. Patients with equivocal CT features for resectability (ie. patients with radiological features approximating the threshold values outlined above) who went on to undergo further staging and subsequent laparotomy were also included in the analysis.

Only patients who underwent both staging laparoscopy and subsequent attempted resection were included in the analysis. This approach was used to retrospectively identify what proportion of staging laparoscopies conducted during the study period were potentially avoidable in the patient group undergoing surgical exploration. Laparoscopic staging included inspection of the peritoneal cavity along with intra-operative ultrasonographic assessment of the liver parenchyma and tumour relationships with local vasculature. Contraindications to laparoscopic staging included the presence of co-morbid disease which would preclude consideration for further investigation, gastric outlet obstruction requiring surgical bypass or significant previous intra-abdominal surgery. Patients with proven metastatic disease from intra-operative biopsy at laparoscopy were excluded from a trial dissection. Patients with equivocal laparoscopic features of resectability who subsequently went on to undergo trial dissection were classified as having potentially resectable disease for the purposes of the study. Laparoscopy and laparoscopic ultrasonography were in routine use throughout the study period.

The operative criteria for unresectable disease at laparotomy were based on the finding of any hepatic or peritoneal metastases proven on frozen section, the presence of vascular encasement or tumour involvement of the portal/superior mesenteric vein precluding the option of local resection. A small proportion of patients underwent venous resection (either sleeve or segmental) in cases exhibiting limited tumour infiltration into the portal or superior mesenteric vein in order to achieve adequate macroscopic tumour clearance where feasible. Extended lymphadenectomy was not routinely undertaken as part of the pancreatoduodenectomy during any part of the

study period. There were no significant differences between surgeons regarding the operative criteria used to determine resectability at laparotomy.

All cases of suspected pancreatic/periampullary cancer (including those with subsequently proven benign disease) were included in the analysis of resectability as the exact origin and histological nature of the primary is commonly unknown at the time of decision-making regarding surgical intervention. Furthermore, the specific tumour origin is frequently not established in patients with metastatic disease identified at laparotomy. Therefore, this overall patient group reflects a more representative sample within which to study the predictive value of biochemical and haematological parameters in a clinically meaningful setting.

A preoperative CA19-9 cut-off value of $\leq$150 kU/l (or $\leq$300 kU/l in cases of concurrent jaundice) was used to stratify patients for predicting tumour resectability. These values were selected in accordance with the previously published literature (Connor et al. 2005). On this basis, an adjusted CA19-9 was calculated for analyses of continuous data by halving the CA19-9 in cases with concurrent obstructive jaundice at the time of CA19-9 estimation (ie. bilirubin levels >35μmol/l). Where concurrent bilirubin levels were unavailable, the unadjusted CA19-9 was used for these analyses. The platelet-lymphocyte ratio was calculated for all patients in whom a preoperative full blood count along with differential white cell count were recorded. Various cut-off values for the platelet-lymphocyte ratio were used to determine the point at which the positive predictive value for resectability was maximised. A value of $\leq$150 was found to represent this point.

Histology reports

Pancreatoduodenectomy specimens were processed and reported according to a previously defined protocol by a Royal College of Pathologists Working Group (Campbell et al. 2002). Specimens were routinely processed according to these criteria for the duration of the study period. The complete histology reports for all cases undergoing pancreatic resection during the study period were obtained from computer records in the Pathology department. A separate Microsoft Access database was constructed to collect and analyse the histology data in detail. The following information and histological tumour characteristics were recorded from the reports:

Type of specimen

Cases were categorised according to whether the main specimen was from a pylorus-preserving partial pancreatoduodenectomy or classical Kausch-Whipple procedure.

Additional procedures undertaken

Details of additional specimens received along with the main specimen were also recorded (eg. cholecystectomy, splenectomy, venous resection (sleeve or segmental), etc).

Site of tumour

Tumours arising from the uncinate process were classified as arising from the pancreatic head. Tumour locations were therefore recorded as either head of pancreas, ampulla or intrapancreatic bile duct. Only adenocarcinomas of confirmed pancreatic origin were analysed.

Histological tumour type

This was based on the WHO classification system for exocrine pancreatic neoplasms (Kloppel et al, 1996). The small number of cases with mucinous adenocarcinoma (non-cystic), signet-ring cell adenocarcinoma, mixed ductal-endocrine carcinoma and undifferentiated adenocarcinoma sub-types arising from the pancreatic head were analysed alongside ductal adenocarcinoma cases.

Size of tumour

The maximum recorded tumour diameter was used as the prognostic variable of interest (measured in millimetres). This value was recorded from the microscopic tumour report for smaller tumours or the macroscopic tumour dimensions for larger tumours.

Tumour differentiation

All tumours were classified as 'well', 'moderately' or 'poorly' differentiated on the basis of a previously defined grading system (Kloppel et al. 1996) - *Table 1.* A differentiation grade was allocated according to the least differentiated area. Therefore, cases reported as 'moderate-to-poorly' differentiated were categorised as poorly differentiated. Similarly, cases reported as 'well differentiated with focal areas of moderate differentiation' were classified as moderately differentiated.

Table 1 - Grading of pancreatic ductal adenocarcinoma

Differentiation	Duct Structures	Nuclei	Mitotic figures*	Mucin production
Well	Well formed	Basal	<5	Marked
Moderate	Some well formed	Loss of polarisation, anisonucleosis	5-10	Variable
Poor	Very irregular or absent	Marked anisonucleosis, clumped chromatin	>10	Minimal

*per 10 high powered fields (1356μm^2)

T stage

T staging was recorded according to the UICC TNM classification for pancreatic, ampullary and biliary adenocarcinoma (*Table 2*).

Nodal staging

Similarly, nodal staging was recorded according to the same UICC TNM classification (*Table 2*). In addition to overall nodal staging, the number of regional lymph nodes containing metastatic adenocarcinoma along with the total number of lymph nodes sampled were recorded in order to calculate the lymph node ratio (ie. the proportion of involved nodes as a ratio of the total number of nodes sampled from the specimen). The distribution of regional lymph nodes was further classified as anterior pancreaticoduodenal, posterior pancreaticoduodenal, inferior, superior, infrapyloric and bile duct where recorded.

Table 2 - UICC TNM classification for pancreatic adenocarcinoma (Sobin et al. 2002)

Pancreas	
T1	Tumour limited to the pancreas, 20mm or less in greatest dimension
T2	Tumour limited to the pancreas, >20mm in greatest dimension
T3	Tumour extends directly into duodenum, bile duct or peripancreatic tissues
T4	Tumour extends directly into stomach, spleen, colon or adjacent large vessels
N0	No regional lymph node metastases
N1	Regional lymph node metastasis present
N1a	Metastasis in single regional lymph node
N1b	Metastasis in multiple regional lymph nodes

Lymph node 8a and 16b sampling

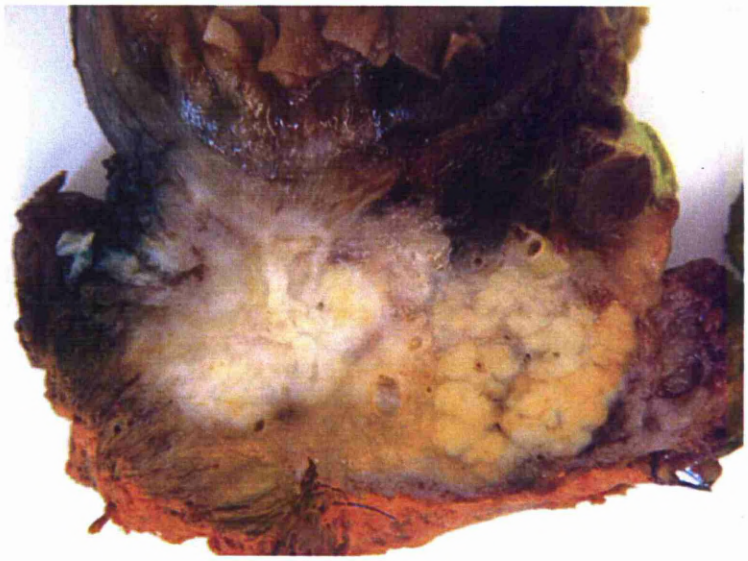
Second order lymph nodes were routinely sampled for resected periampullary adenocarcinoma. Nodes from the anterior aspect of the common hepatic artery (LN8a) and retroperitoneal nodes posterior to the head of the pancreas (LN16b) were most frequently sampled. Nodal stations were identified intra-operatively according to the Japan Pancreas Society (JPS) classification (Japan Pancreas Society, 2003). Routine extended lymphadenectomy was not employed at any point during the study period.

Resection margins

All pancreatoduodenectomy specimens were serially sliced axially and histopathology reporting was conducted according to the Royal College of Pathologists Minimum Dataset for pancreatic and periampullary adenocarcinoma (Campbell et al. 2002). The reporting criteria in the Dataset were routinely used both before and after their publication in 2002. *fig.1* demonstrates an example of an axial slice through the head

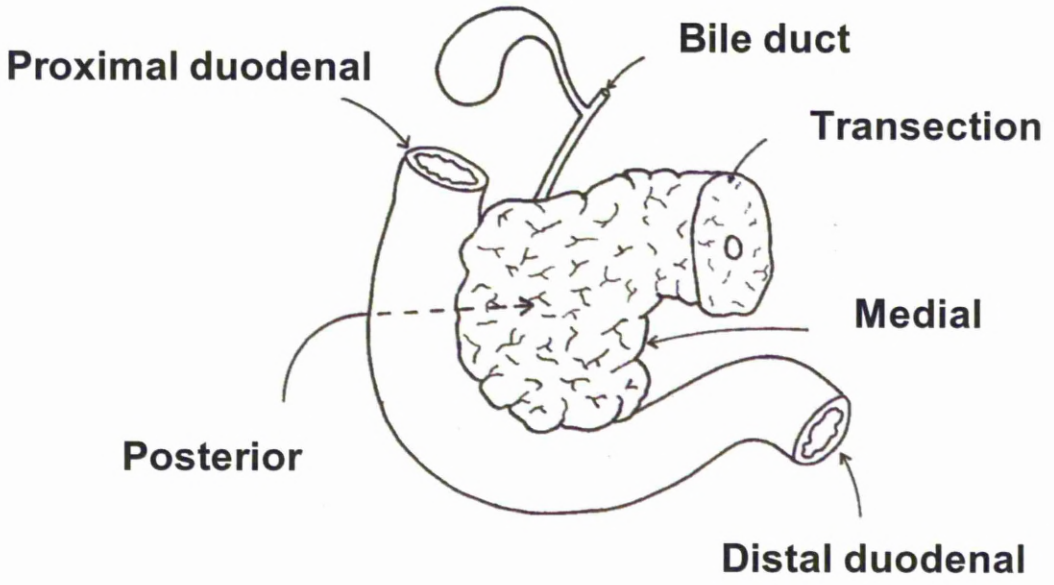
of the pancreas with adenocarcinoma extending close to the posterior (green) resection margin and clear of the medial (orange) resection margin. A lymph node is seen within the anterior pancreatoduodenal groove (yellow paint).

fig.1 - Macroscopic photograph demonstrating example of axial slicing of pancreatoduodenectomy specimen.



The guidelines recommend that the status of six discrete resection margins be documented by the reporting pathologist - the pancreatic transection margin, the medial (or superior mesenteric vessel) margin, the posterior margin, the proximal duodenal (or gastric) margin, the distal duodenal margin and the common bile duct margin (*fig.2*).

fig.2 - Principal resection margin locations in pancreatoduodenectomy specimens.



The presence or absence of reported tumour involvement at one or more resection margins was recorded by the reporting pathologist and all cases with microscopic tumour involvement within 1 mm, without directly breaching one or more margins were also documented. In all cases reported as R0, the histopathology slides were retrieved and microscopically reassessed alongside a single consultant pathologist (FC) in order to confirm R0 classification. *fig.3* illustrates an example of an 'equivocal' resection margin where tumour can be seen to extend to within 1mm, without directly breaching the margin itself. *fig.4* shows an example of direct tumour involvement at a painted resection margin.

fig.3 - Light micrograph demonstrating 'equivocal' microscopic tumour involvement (ie. within 1mm of a resection margin without directly breaching the painted surface).

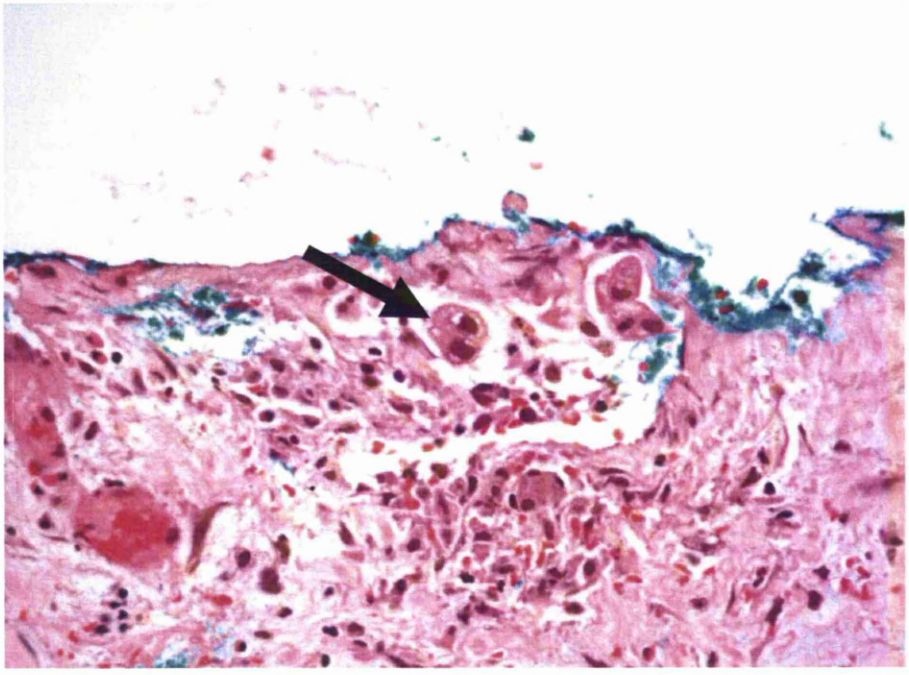
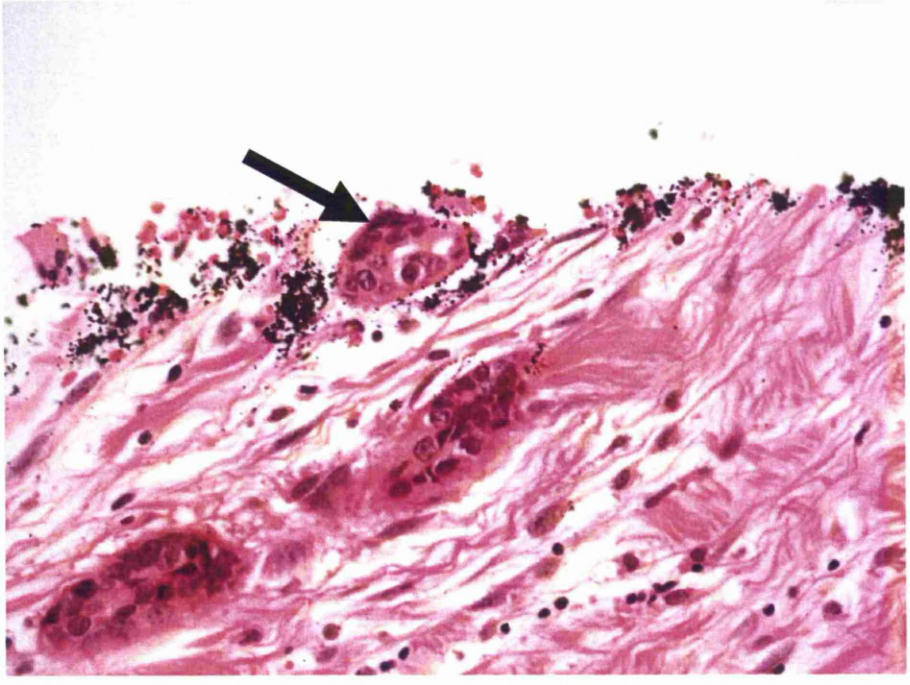


fig.4 - Light micrograph demonstrating direct microscopic tumour involvement directly breaching a painted resection margin.



Isolated tumour involvement of the anterior capsule of the pancreatic specimen was not considered as an R1 resection as part of this study. Similarly, the presence of PanIN-3 at an otherwise negative transection margin was not considered an R1 resection. No cases were classified as R1 exclusively on the basis of perineural invasion at a resection margin. Similarly, nodal involvement at a resection margin did not constitute an R1 classification in the absence of direct tumour involvement. No R2 resections were reported in this series of patients.

Vascular and perineural invasion

The presence or absence of perineural invasion (ie. tumour invasion involving one or more intrapancreatic nerve bundles) on microscopic assessment of the specimen documented by the reporting pathologist was recorded. Similarly, the presence or absence of vascular invasion (ie. tumour invasion involving one or more intrapancreatic blood vessels) was also recorded.

Statistics

Median, interquartile range and 95% confidence intervals were used to describe continuous data. Comparisons of grouped continuous data were conducted using Mann-Whitney U testing or Krukal-Wallis testing where data from more than two groups were included. Correlation between two continuous variables was evaluated using Spearman's rank correlation. Proportional differences in categorical data were analysed using Chi-squared testing with Pearson's correction where cell frequency was greater than 10 and Yates' correction in cases where cell frequency was 5 to 10. Fisher's exact test was used in cases where cell frequency was <5. Logistic regression was used to investigate the effect of one or more continuous or categorical independent variables on a categorical dependent variable. Receiver operating characteristic curves were generated to compare the predictive value of two parameters in determining disease resectability.

Univariate survival data were analysed using Kaplan-Meier cumulative survival curves with log rank (Mantel-Cox) testing to demonstrate differences in survival between two or more groups from a single categorical prognostic parameter. Where a continuous prognostic variable was analysed, univariate analysis was primarily conducted with Cox proportional hazards regression on a continuous basis. Because of the wide range in preoperative CA19-9 levels recorded, this prognostic variable was normalised for Cox modelling by logarithmic transformation (ie. lnCA19-9). Only prognostic variables of (or approaching) univariate significance were selected for inclusion in subsequent multivariate survival analyses.

Hazard ratios for continuous variables included in a Cox analysis reflect the proportional increase in relative risk of death (hazard) with each incremental increase in the continuous prognostic variable of 1 unit. For instance, a hazard ratio of 1.004 reflects a regression coefficient of 0.004 (ie. $e^{0.004} = 1.004$). Therefore, the relative hazard associated with a comparative difference of 200 units for this variable would be $e^{(200x0.004)} = 2.226$. The Chi-squared statistic gives a further indication of the strength of the relationship between the prognostic variable and survival. Proportionality was checked for all variables prior to inclusion for Cox regression by reviewing the log cumulative hazard plots for each variable when categorised.

Dichotomizing a continuous prognostic variable is associated with potential significant bias due to an inflated type I error rate along with the fact that significance can be variably seen when using a number of different cut-off points for the parameter of interest. This frequently results in an overestimated significance level on univariate analysis along with a disproportionate weighting on subsequent multivariate analysis (Altman et al. 1994). In order to generate Kaplan-Meier cumulative survival curves for continuous prognostic variables, cut-off values were selected either on the basis of the normal reference range for the parameter of interest or according to the maximum degree of risk stratification allowed (three groups where possible). It should be pointed out that none of the reported survival relationships for continuous prognostic variables were reliant on any data-driven cut-off points as all Cox analyses for such variables were conducted on a continuous basis. Hence, cut-off points were only used to graphically illustrate the recorded survival trends.

Statview version 5 (ISAS Instituite, Cary, NC, USA) and Microsoft Excel (Microsoft Office 2002) were used to perform the various statistical functions. SPSS version 14.0 (SPSS Inc. 2005) was used to generate the ROC curves.

2.2. RESULTS & DISCUSSION

For patients undergoing pancreatoduodenectomy for tumours arising from the pancreatic head or uncinate process, the following histological classifications recorded by the reporting pathologist were included for analysis:

- pancreatic ductal adenocarcinoma (n=151)
- 'adenocarcinoma' (n=8)
- mucinous (non-cystic) adenocarcinoma (n=4)
- signet-ring cell carcinoma (n=1)
- mixed ductal-endocrine carcinoma (n=1)
- anaplastic (n=1)
- **Total = 166**

This group of cases was collectively analysed and is subsequently referred to as 'pancreatic ductal adenocarcinoma' for the purposes of this study.

All figures with the suffix 'A' (eg. *fig.2A*) indicate non-significant survival analyses and are included in Appendix A for reference.

Publications arising from the content of the following analyses are listed in Appendix B. There are some marginal differences in the results when comparing the survival analyses in this thesis with the results contained in the various publications. This is a result of the fact that the analyses within this thesis included an additional cohort of resections undertaken between 2006 and 2007 when compared with some of the published papers along with the fact that the survival data was updated at the end of this study period.

2.2.1. Summary survival data

Study group

166 patients identified from the database underwent pylorus-preserving pancreatoduodenectomy or classical Kausch-Whipple procedures for pancreatic ductal adenocarcinoma during the study period. This group comprised 94 males (56.6%) and 72 females (43.4%) with a median age of 66.4 (IQR = 60.8 to 72.8) years. 132 patients (79.5%) died during the follow-up period with 34 (20.5%) censored cases. The censored cases had a median follow-up time of 15.3 (IQR = 10.3 to 29.0) months. The median survival time for the overall group was 13.9 (95% CI = 12.4 to 16.1) months. There were 4 deaths within 30 days of surgery (2.4%) and 7 deaths prior to discharge from hospital (4.2%). *Fig.1* demonstrates the survival distribution for the overall patient group.

fig.1 - Kaplan-Meier cumulative survival curve for overall pancreatic ductal adenocarcinoma patient group (crosses indicate censored cases).

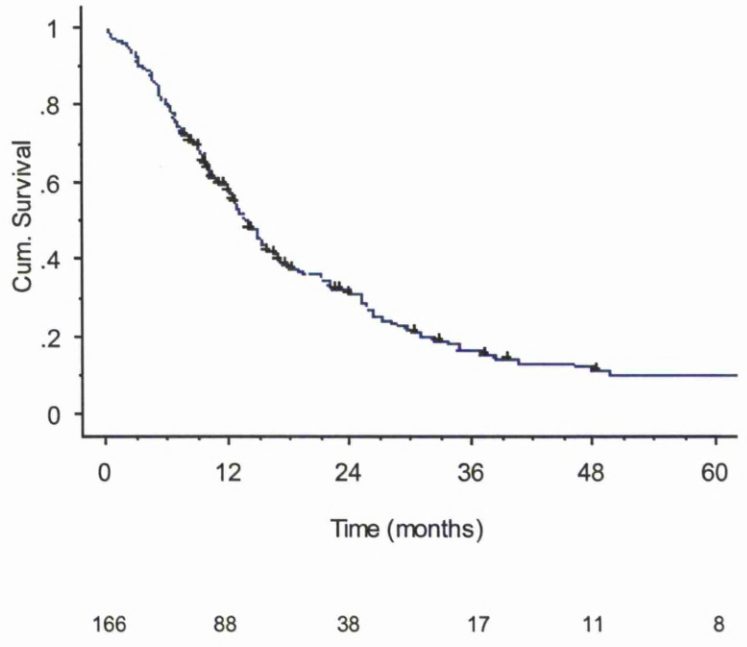


Table 1 - Summary table of clinico-pathological characteristics for resected pancreatic ductal adenocarcinoma patients.

Total no. of patients identified	166
Gender:	
Male	94 (56.6%)
Female	72 (43.4%)
Age: median (IQR)	66.4 (60.8 to 72.8) years
Median overall survival (95% CI)	13.9 (12.4-16.1) months
No. of censored cases	34 (20.5%)
Median follow-up time for censored cases (IQR)	15.3 (10.3-29.0) months
Type of surgery:	
PPPD	149 (89.8%)
Classical Kausch-Whipple	17 (10.2%)
Venous resections	10 (6.0%)
30-day mortalities	4 (2.4%)
In-patient mortalities	6 (3.4%)
Neoadjuvant therapy received	3 (1.8%)
Adjuvant therapy received	51 (30.7%)
Chemotherapy	45
Chemoradiotherapy	6
Tumour histology	
Median tumour size (IQR) - n=160	30 (22-38) mm
T stage: (n=165)	
1	7 (4.2%)
2	17 (10.3%)
3	137 (83.0%)
4	4 (2.4%)
Tumour differentiation: (n=165)	
well	25 (15.2%)
moderate	85 (51.5%)
poor	55 (33.3%)
Nodal status (n=166):	
negative	27 (16.4%)
positive	139 (83.6%)
Median lymph node ratio in N+ve cases (IQR): (n=135)	0.23 (0.14-0.37)
Resection margin status: (n=163)	
negative	35 (21.5%)
positive	128 (78.5%)

NB. Histological data were incomplete for a small number of cases. IQR = interquartile range. CI = confidence interval.

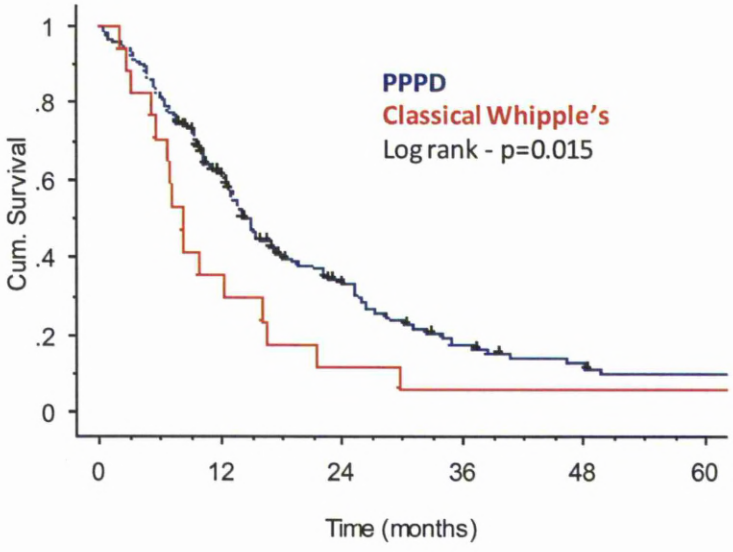
2.2.2. Clinical / patient-related factors

No significant difference in median survival was recorded when comparing males (13.3 (95% CI = 10.4 to 16.6) months) and females (14.3 (95% CI = 12.1 to 22.6) months) - log rank, p = 0.289 - *fig.2A* (Appendix A). Similarly, Cox regression using age as a continuous prognostic variable demonstrated that there was no significant relationship between increasing age and overall survival in patients with resected pancreatic cancer (HR = 1.000 (95% CI = 0.970 to 1.021), p = 0.970).

PPPD vs classical Kausch-Whipple's

149 patients (89.8%) who underwent PPPD had a median survival of 14.3 (95% CI = 12.8 to 17.4) months) compared with 8.2 (95% CI = 5.5 to 16.1) months in 17 (10.2%) who underwent a classical Kausch-Whipple procedure - log rank; p = 0.015 - *fig.3*.

fig.3 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to type of surgery.



No. at risk						
PPPD	149	82	36	16	10	7
Classical	17	6	2	1	1	1

This finding is concordant with published results from ESPAC-1 (Bassi et al, 2005) and might potentially be explained on the basis that patients with less favourable histological tumour characteristics at operation were more likely to require classical Kausch-Whipple procedures than those undergoing PPPD. *Table 1* demonstrates a breakdown of tumour histology according to the type of resection.

Table 1 - Breakdown of tumour histology according to type of pancreatoduodenectomy undertaken.

	PPPD (n=159)	**Classical Kausch-Whipple (n=17)**	**p-value**
Median tumour size (IQR) mm	30 (22 – 38)	30 (25 – 45)	0.743*
Nodal status:			
Negative	24	3	0.999
Positive	125	14	
Differentiation:			
Well/moderate	103	7	**0.023****
Poor	45	10	
T stage:			
1 & 2	22	2	0.999
3 & 4	126	15	
Resection margin status:			
Negative	34	1	0.197
Positive	113	15	
Adjuvant chemotherapy:			
No	106	15	0.160
Yes	43	2	

Quoted p-values for Fisher's exact except:

*p-value for Mann-Whitney test

**p-value for χ^2 test

Venous resection

A total of 10 patients (6.0%) underwent venous resection (either sleeve or segmental) as part of their pancreatoduodenectomy in cases exhibiting intraoperative tumour infiltration into the portal or superior mesenteric vein in order to achieve adequate macroscopic tumour clearance. Patients undergoing venous resection had a median survival of 17.4 (95% CI = 10.4 to 22.1) months compared with 13.8 (95% CI = 12.3 to 15.9) months for the remaining group - log rank; p = 0.902 - *fig.4A.*

Survival according to study period

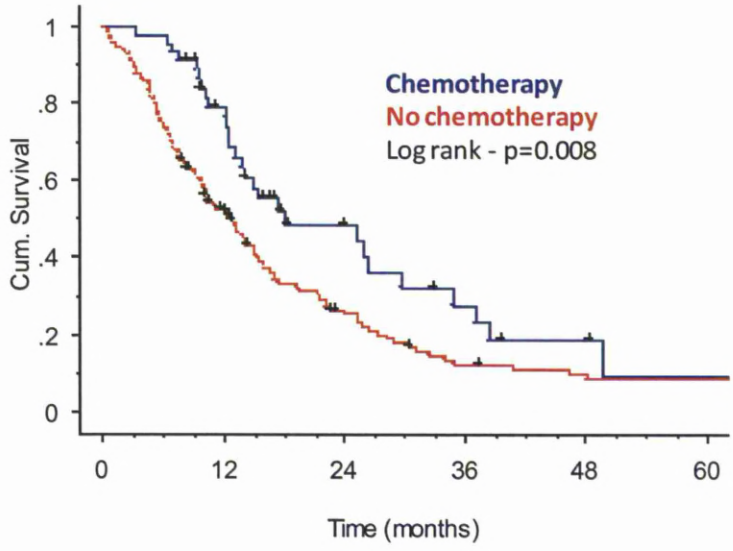
An analysis was undertaken to identify whether there was any trend towards more favourable survival in patients operated on during the second half of the study period when compared with the first. 62 patients (37.3%) who underwent surgery between 1997 and 2002 had a median survival of 12.8 (95% CI = 8.5 to 17.0) months while 104 patients (62.7%) undergoing surgery between 2003 and 2007 had a median survival of 14.2 (95% CI = 12.6 to 17.4) months - log rank; p = 0.647 - *fig.5A.*

This result suggests that there was no significant difference in survival according to timing of surgery during the course of the study period. A significantly greater proportion of patients (40/104 - 38.5%) went on to receive adjuvant therapy in the second half of the study period when compared with the first half (11/62 - 17.7%) - χ^2 = 7.83; p = 0.005. Furthermore, a greater proportion of censored cases are inevitably present during the second half of the study (due a shorter overall follow-up period) when compared with the first. Despite these potential confounding factors, the data suggest that there was no significant overall trend to indicate more favourable survival outcomes in patients undergoing surgery in more recent years.

Adjuvant therapy

Only 3 patients (1.8%) received some form of neoadjuvant therapy - these were cases initially staged as locally advanced disease who were subsequently down-staged and went on to have a successful resection. 51 patients (30.7%) received adjuvant therapy following surgery - 45 received chemotherapy (either 5-FU or gemcitabine) and 6 received chemoradiotherapy (5-FU based - ESPAC-1 protocol). Patients receiving adjuvant chemotherapy had a more favourable median survival (18.2 (95% CI = 13.8 to 29.7) months) when compared with patients who did not receive chemotherapy (12.8 (95% CI = 9.9 to 15.4) months) - log rank; p = 0.008 - *fig.6*. The limited number of patients receiving chemoradiotherapy had comparable survival times to patients who received no subsequent adjuvant therapy (log rank; p = 0.754) - *fig.7A*. These results largely mirror the findings from ESPAC-1 (Neoptolemos et al. 2004).

fig.6 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to adjuvant chemotherapy.



No. at risk						
Chemo	45	30	13	6	3	1
No Chemo	121	58	25	11	8	7

2.2.3. *Discussion*

The overall survival time recorded for the entire study group is comparable with reported median survival times recorded for resected pancreatic ductal adenocarcinoma patients from previous meta-analyses of adjuvant therapy trials (Stocken et al, 2005) and published SEER data (Slidell et al, 2008) given that around one in three patients received adjuvant chemotherapy. The marginal gender preponderance of males is representative of the overall epidemiological distribution of pancreatic adenocarcinoma incidence. The low incidence of operative mortalities during the overall study period is also in keeping with operative results from high-volume specialist pancreatic units (Neoptolemeos et al, 1997).

Neither age nor gender were demonstrated to have any impact on overall survival and this observation is a characteristic finding from previous literature reporting survival outcomes following resection for pancreatic cancer (Garcea et al, 2008). The small proportion of patients for whom venous resection was required as part of their surgery failed to exhibit any adverse overall survival when compared with patients undergoing standard resections. Venous resection is appropriate for a sub-group of tumours exhibiting involvement of the hepatic portal or superior mesenteric vein providing adequate macroscopic tumour clearance can be achieved. Although venous resection has the potential to maximise resectability, it has not been demonstrated to have any impact on survival (Bold et al, 1999; van Geenan et al, 2001; Nakagohri et al, 2003) and the findings from the current study are in keeping with this.

A previous meta-analysis of seven randomised controlled trials comparing outcomes following pylorus-preserving pancreatoduodenectomy vs. classical Whipple

procedures failed to demonstrate any significant differences in operative mortality, morbidity or overall survival (Diener et al, 2008). However, the results did suggest longer operative times and greater intraoperative blood loss in patients undergoing classical Whipple procedures. Pylorus-preserving pancreatoduodenectomy was considered as the procedure of choice for resectable tumours of the pancreatic head throughout the duration of the study period for the current patient cohort. The limited number of patients who required classical Whipple procedures were found to have a poorer overall survival and this observation is likely to be explained on the basis that those patients for whom classical Whipple procedures were performed exhibited more unfavourable tumour characteristics at laparotomy necessitating a more extensive resection in order to achieve adequate oncological clearance. The breakdown of tumour histology according to type of surgery was indicative of this fact with a significantly greater proportion of poorly differentiated tumours in patients requiring classical Whipple procedures. Because of this finding, the type of pancreatoduodenectomy was not included as a covariate in subsequent multivariate analyses.

Patients receiving adjuvant chemotherapy were found to have a significantly greater median survival when compared with those patients who received no adjuvant chemotherapy and the small number of patients who received adjuvant chemoradiotherapy. Most patients who went on to receive adjuvant therapy following surgery did so as part of either ESPAC-1 or ESPAC-3. The observation that chemotherapy conferred a significantly improved survival mirrors the findings from ESPAC-1, a randomized controlled trial conducted between 1994 and 2000, recruiting 541 patients with resected pancreatic ductal adenocarcinoma from 61 centres in 11

European countries. 285 patients were formally randomised into a two-by-two factorial study design whereby participants received either chemoradiotherapy, chemotherapy, chemoradiotherapy with follow-on chemotherapy or observation. Chemotherapy consisted of bolus 5-FU and folinic acid given over 5 consecutive days every 28 days for six cycles. The CRT regimen consisted of a 20 Gy dose given in 10 daily fractions over a two week period along with a bolus of 5-FU. The mature trial results were published in 2004 (Neoptolemos et al, 2004). A total of 147 patients randomized to receive chemotherapy had a median survival of 20.1 months compared with 15.5 months in 142 patients who did not receive chemotherapy (p=0.009). CRT was shown to be associated with a deleterious survival outcome with 145 patients assigned to receive CRT exhibiting a median survival of 15.9 months compared with 17.9 months in 144 patients not receiving CRT (p=0.05). In order to evaluate the findings of ESPAC-1 alongside the existing evidence base for use of adjuvant therapy in pancreatic cancer, a meta-analysis of all randomized adjuvant therapy trials was undertaken and published in 2005 (Stocken et al, 2005). This analysis included the survival data from ESPAC-1, GITSG (Kalser et al, 1985; Gastrointestinal Tumour Study Group, 1987), EORTC (Klinkenbijl et al, 1999), and two studies evaluating adjuvant chemotherapy regimens vs. observation (Bakkevold et al, 1993; Takada et al, 2002). 875 patients were included in the overall analysis. The findings confirmed that administration of CRT was associated with no survival advantage while patients receiving chemotherapy exhibited significantly more favourable survival times.

Oettle et al published the results of the CONKO-01 study in 2007, a multicentre randomised controlled trial evaluating adjuvant gemcitabine versus no adjuvant therapy in 368 patients undergoing radical resection for pancreatic ductal

adenocarcinoma. A significantly improved disease free interval in the adjuvant treatment arm (13.4 months) over the observation arm (6.9 months) was demonstrated. However, the difference in median overall survival rates between the two groups did not reach significance (22.1 vs 20.2 months respectively - p=0.06). Despite this finding, the 5-year survival rates associated with the treatment and control groups in this study (22.5% and 11.5% respectively) are consistent with those reported in ESPAC-1 (21% and 8% respectively). The more recently published results from ESAPC-3 (Neoptolemos et al, 2010) failed to demonstrate any significant survival benefit associated with use of adjuvant gemcitabine when compared with 5-FU and folinic acid.

Given the significant impact of adjuvant chemotherapy on postoperative survival in the current patient cohort along with the existing evidence base, this factor was included as a covariate in all subsequent multivariate analyses of prognostic factors. No attempt was made to conduct any sub-group analysis based on type of chemotherapy received (ie. 5-FU vs. gemcitabine).

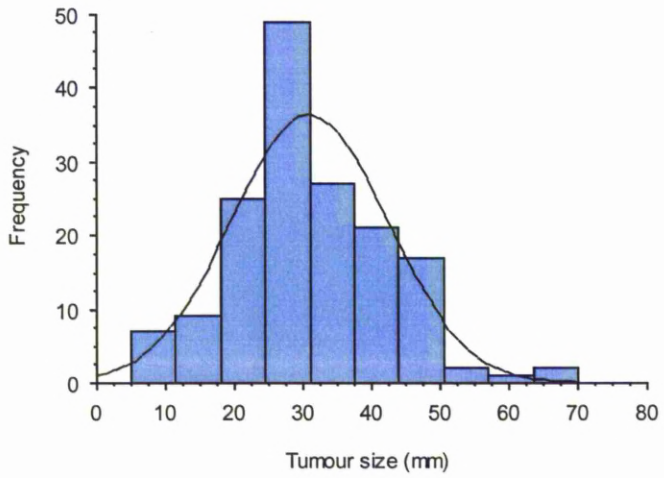
The proportion of patients receiving adjuvant chemotherapy following resection in the overall study group (30%) was lower than what might be expected given the above evidence base. However, the results indicate that the proportion of patients receiving chemotherapy increased during the course of the 10-year study period. This pattern may in part be explained by evolving oncological practices during this time with less restrictive selection criteria applied to assess patient fitness for adjuvant therapy.

2.3. Histological prognostic factors

2.3.1. *Tumour size*

The maximum recorded tumour diameter was obtained for 160 out of 166 cases. The median recorded tumour diameter was 30 (IQR = 22 to 38) mm for these cases. *Fig.8* demonstrates that the distribution of tumour size for resected pancreatic ductal adenocarcinoma cases approximated a normal distribution.

fig.8 - Distribution of tumour size for resected pancreatic ductal adenocarcinoma cases (n=160).

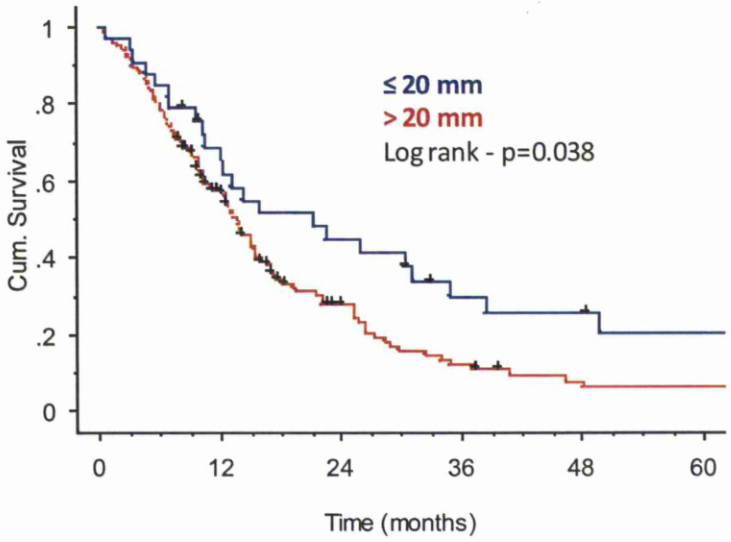


The univariate prognostic influence of tumour size on overall survival was assessed in 160 evaluable patients. The patient group was comparatively analysed by modelling tumour size as both a continuous prognostic variable and by dichotomising cases into one of two groups based on whether the tumour size was ≤ 20mm or > 20mm (*fig.9*). This standard cut-off value was selected on the basis of existing pancreatic literature and the fact that the criteria for histological T stage (ie. T1 vs. T2) is also based on a cut-off size of > 20 mm. Cox proportional hazards regression was used to generate hazard ratios for both analyses (*Table 2*). The results from this analysis demonstrate a significant relationship between increasing tumour size and poorer survival on a univariate basis.

Table 2 - Univariate prognostic significance of tumour size - Cox proportional hazards (n=160).

	Median survival (95% CI)	**Hazard ratio (95% CI)**	χ^2	**p-value**
Tumour size:				
≤20mm (n=33)	22.6 (12.4 to 32.4)	-		-
>20mm (n=127)	13.3 (11.1 to 15.4)	1.588 (1.021 to 2.471)	4.213	**0.040**
Tumour size: (continuous)	-	1.020 (1.005 to 1.036)	6.619	**0.010**

fig.9 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to tumour size ≤20mm.

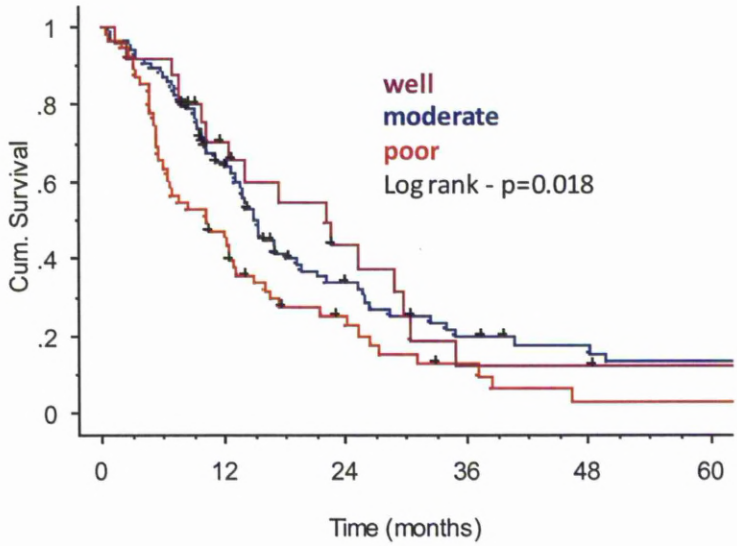


No. at risk						
≤20 mm	33	20	13	7	6	4
>20 mm	127	65	24	10	5	4

2.3.2. *Tumour differentiation*

Tumour differentiation was recorded in 165 of the 166 pancreatic ductal adenocarcinoma cases. 25 cases (15.2%) had a well differentiated tumour, 85 cases (51.5%) had a moderately differentiated tumour and 55 cases (33.3%) had a poorly differentiated tumour. *fig.10* demonstrates the survival curves associated with each of the tumour grades. *Table 3* outlines the Cox proportional hazards analysis and median survival times for the three differentiation types.

fig.10 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to tumour differentiation (3 groups).



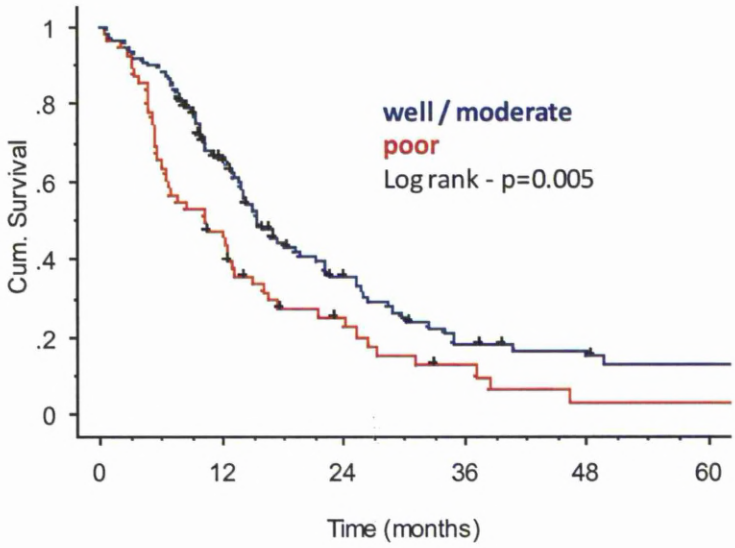
No. at risk						
well	25	14	7	2	2	1
moderate	85	49	21	11	8	6
poor	55	25	10	4	1	1

Table 3 - Univariate prognostic significance of tumour differentiation - Cox proportional hazards.

	Median survival (95% CI)	**Hazard ratio (95% CI)**	χ^2	**p-value**
Differentiation (3groups):				
Well (n=25)	22.1 (12.5 to 29.7)	-	-	-
Moderate (n=85)	15.4 (13.2 to 19.2)	1.048 (0.620 to 1.772)	0.031	0.860
Poor (n=55)	10.4 (6.4 to 13.3)	1.735 (1.007 to 2.988)	3.948	**0.047**
Differentiation (2 groups):				
well / moderate (n=110)	15.5 (13.8 to 21.3)	-	-	-
poor (n=55)	10.4 (6.4 to 13.3)	1.673 (1.167 to 2.399)	7.828	**0.005**

Given the small numbers of well differentiated tumours observed in the pancreatic ductal adenocarcinoma patients, along with the finding that there was no significant difference in overall survival between the well and moderately differentiated tumour groups (Cox; p=0.860), cases with these two tumour grades were grouped together for subsequent analyses. Well/moderately differentiated tumours (n=110) were associated with a significantly more favourable median survival when compared with poorly differentiated tumours (*fig.11*) - log rank; p = 0.005.

fig.11 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to tumour differentiation (2 groups).

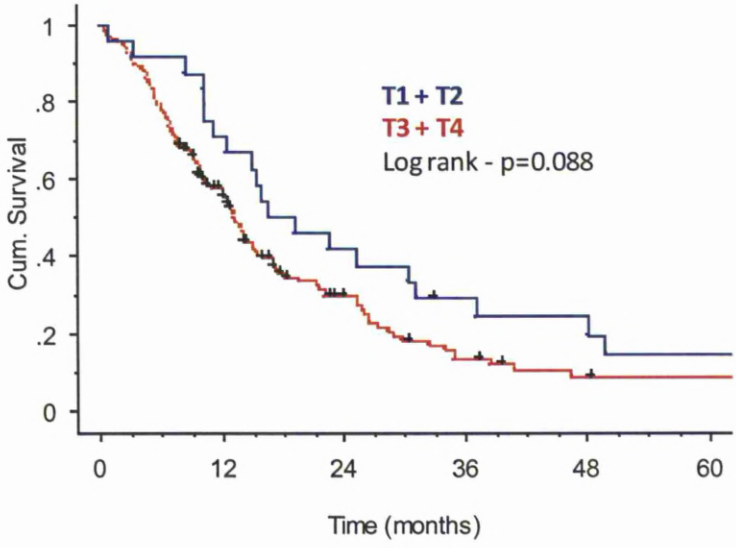


No. at risk						
well / mod	110	63	28	13	10	7
poor	55	25	10	4	1	1

2.3.3. *T stage*

T stage was recorded for 165 out of the 166 pancreatic ductal adenocarcinoma cases. 7 patients (4.2%) had a T1 tumour, 17 patients (10.3%) had a T2 tumour, 137 patients (83.0%) had a T3 tumour and 4 patients (2.4%) had a T4 tumour. Due to the small numbers of T1 and T4 tumours, T1/T2 tumours and T3/T4 tumours were grouped together for analysis. *fig.12* demonstrates the survival curves for these cases. T1/T2 tumours were associated with a median survival of 16.6 (95% CI = 12.5 to 31.2) months compared with 13.2 (95% CI = 10.5 to 15.4) months for T3/T4 tumours (Cox; HR = 1.503 (95% CI = 0.937 to 2.409), p=0.091).

fig.12 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to T stage (2 groups).



No. at risk						
T1 + T2	24	17	10	6	5	3
T3 + T4	141	70	27	11	6	5

2.3.4. *Nodal status*

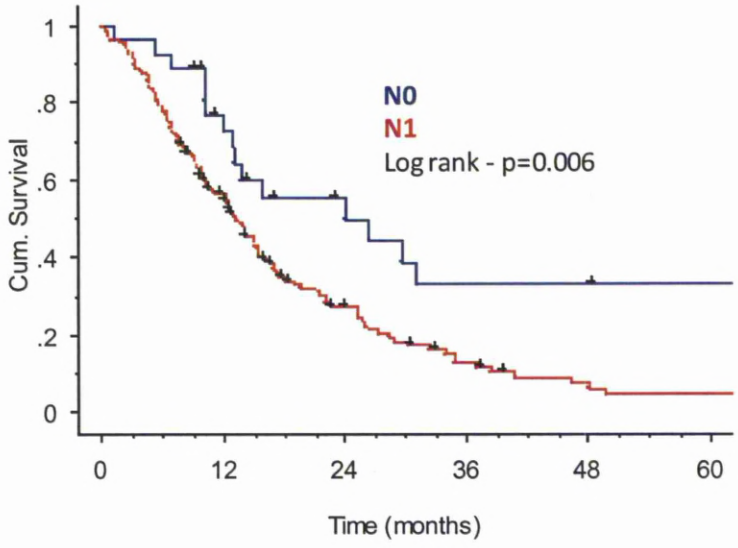
Nodal status (ie. N0, N1a or N1b) was recorded in all 166 patients with pancreatic ductal adenocarcinoma. There were 27 (16.3%) N0 patients and 139 (83.7%) N1 patients. The number of lymph nodes containing metastatic adenocarcinoma was recorded in 164 cases and the total number of sampled lymph nodes was recorded in 163 patients. Hence, the lymph node ratio could be calculated for 163 patients from the overall group. The total number of sampled nodes included both regional peripancreatic lymph nodes along with second order nodes sampled during surgery (ie. LN8a, LN16b). *Table 4* outlines the distribution of lymph node characteristics from within this group of patients.

Separate analyses investigating the prognostic relevance of overall nodal status, lymph node ratio and lymph node yield were conducted. *fig.13* demonstrates the survival curves for pancreatic ductal adenocarcinoma cases according to overall nodal status (ie. positive vs negative).

Table 4 - Lymph node characteristics of resected pancreatic adenocarcinoma specimens.

Overall nodal status:	
negative	27 (16.3%)
positive	139 (83.7%)
Median number of sampled lymph nodes (IQR)	17 (12 to 25)
Median number of positive lymph nodes in N1 cases	4 (2 to 6)
Number of cases with ≥ 12 nodes sampled	125 (76.7%)
Median lymph node ratio in N1 cases (IQR)	0.23 (0.14 to 0.37)

fig.13 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to overall nodal status (n=166).



No. at risk						
N0	27	18	10	6	5	5
N1	139	70	28	11	5	3

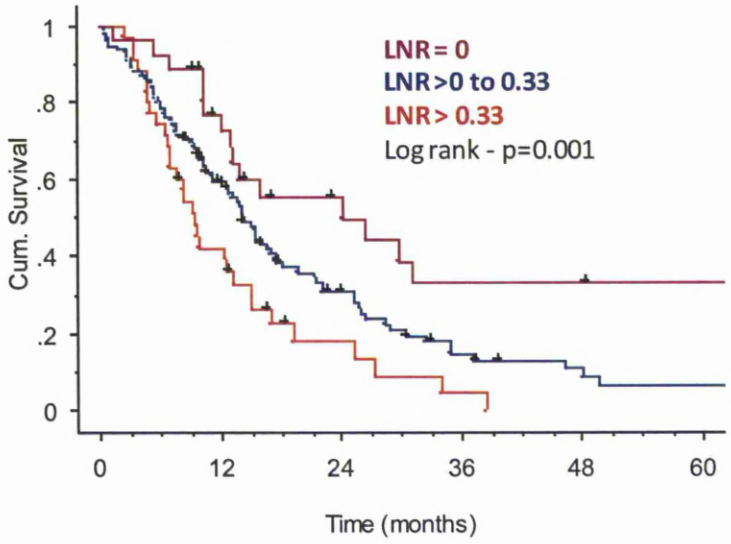
Patients with nodal involvement had a median survival of 13.3 (95% CI = 10.5 to 15.5) months compared with 24.1 (95% CI = 13.0 to 70.7) months for node negative patients (Cox; HR = 2.016 (95% CI = 1.221 to 3.357), p=0.007).

The lymph node ratio was investigated to identify whether this index represents a more informative prognostic marker than overall nodal status. The median lymph node ratio recorded for node positive patients (n=136) was 0.23 (IQR = 0.14 to 0.37). As with tumour size, the prognostic value of the lymph node ratio was investigated both as a categorical and continuous variable. *Table 5* outlines the results of the Cox proportional hazards analysis. *fig.14* demonstrates the Kaplan-Meier cumulative survival curves stratified by three lymph node ratio groups.

Table 5 - Univariate prognostic significance of lymph node ratio (LNR) - Cox proportional hazards (n=163).

	Median survival (95% CI)	**Hazard ratio (95% CI)**	χ^2	**p-value**
LNR (3groups):		-	12.76	**0.002**
0 (n=27)	24.1 (13 to 70.7)	-	-	-
0 to 0.33 (n=101)	14.3 (11.3 to 17.4)	1.819 (1.079 to 3.069)	5.04	**0.025**
>0.33 (n=35)	9.5 (6.9 to 13.3)	2.969 (1.624 to 5.425)	12.51	**<0.001**
LNR:				
continuous (n=163)	-	4.510 (1.938 to 10.494)	12.22	**<0.001**

fig.14 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients stratified by lymph node ratio (n=163).



No. at risk						
N0	27	18	10	6	5	5
LNR >0 to 0.33	101	53	23	9	5	3
LNR > 0.33	35	14	4	1	0	0

An increasing proportion of tumour involved lymph nodes sampled from the surgical specimen was found to closely correlate with poorer survival. These results indicate that the lymph node ratio provides superior prognostic information when compared with overall nodal status. As with tumour size, the lymph node ratio was included as a continuous covariate for subsequent multivariate analyses on the basis of these findings.

The lymph node yield was also investigated as a potential prognostic index. Previous studies have suggested that pancreatoduodenectomy cases with fewer than 12 sampled lymph nodes are associated with a poorer survival outcome than those with more than 12 sampled nodes, irrespective of metastatic nodal involvement (Slidell et al, 2008). On this basis, a further analysis was conducted using the number of sampled nodes as a continuous prognostic covariate for univariate Cox regression in order to assess the relationship between lymph node yield and survival.

The median number of lymph nodes sampled in node positive patients was 19 (IQR = 13 to 26). This compared with 15 (IQR = 9 to 18) for node negative patients. A logistic regression analysis was conducted using the number of sampled nodes as an independent variable and nodal status (ie. positive vs negative) as the dependent variable. This result demonstrated an odds ratio of 1.085 (95% CI = 1.025 to 1.148) - p = 0.005 (n=163). This suggests that the likelihood of identifying metastatic lymph node involvement increases proportionally as the number of sampled nodes increases. When modelling the number of sampled nodes as a continuous variable for Cox regression in the overall patient group there was no significant relationship between lymph node yield and survival (HR = 1.010 (95% CI = 0.992 to 1.020), p = 0.269).

This was equally true when only analysing the N0 group (HR = 0.980 (95% CI = 0.923 to 1.040), p = 0.501.

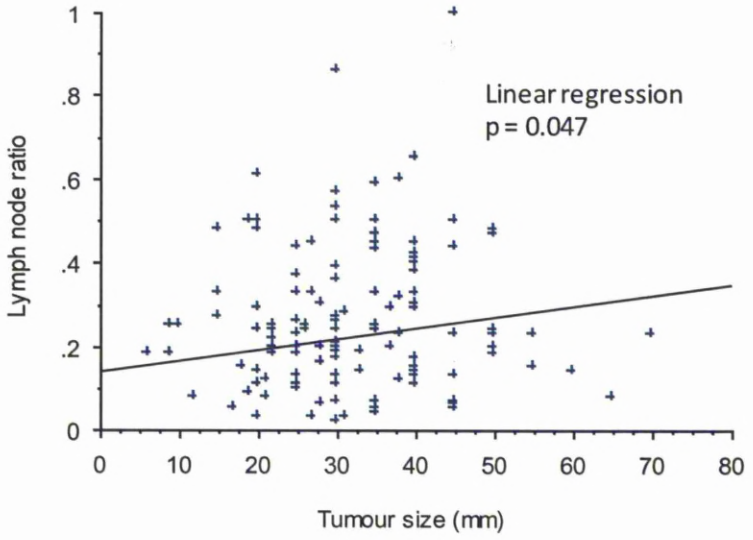
An additional analysis was conducted in order to identify whether reporting pathologists exhibited a tendency for a greater number of lymph nodes to be sampled from pancreatoduodenectomy specimens with less favourable histological tumour characteristics in order to provide an alternative explanation for this observation. *Table 6* outlines the median lymph node yield according to the various histological sub-groups. There was no significant correlation between tumour size and the corresponding number of sampled lymph nodes (Spearman; rho = 0.140, p=0.090). These results did, however, indicate a significant trend towards a greater lymph node yield in more locally infiltrative tumours.

Table 6 - Relationship between tumour histology and lymph node yield.

	Median lymph node yield (IQR)	**p-value**
Resection margin status:		
R0 (n=35)	17 (10 to 22)	0.171
R1 (n=126)	18 (14 to 26)	(Mann-Whitney)
T stage:		
T1 (n=7)	5 (2.5 to 11)	**<0.001**
T2 (n=17)	10 (7 to 19.5)	(Kruskal-Wallis)
T3/T4 (n=141)	19 (14 to 26)	
Tumour differentiation:		
Well (n=23)	17 (13 to 24)	0.883
Moderate (n=85)	18 (13 to 26)	(Kruskal-Wallis)
Poor (n=55)	17 (12 to 25)	

Despite the finding that there was no significant relationship between tumour size and the corresponding number of sampled lymph nodes identified within the pancreatoduodenectomy specimen, there was a significant relationship between increasing tumour size and lymph node ratio - linear regression; R = 0.160, p = 0.047 - *fig.15*.

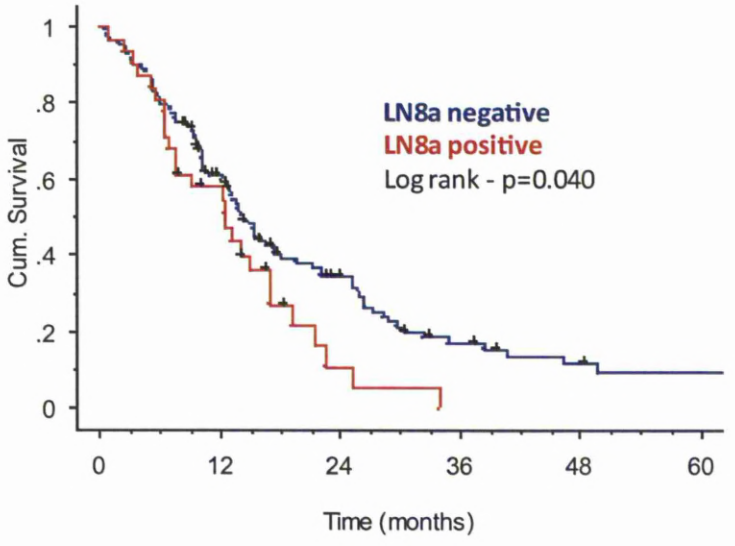
fig.15 - Scattergram to demonstrate trend towards increasing lymph node ratio in larger tumours.



2.3.5. *Lymph node 8a status*

Lymph node 8a was sampled in 140 cases and was found to be positive in 31 (22.1%). Patients with a positive lymph node 8a status had a median survival of 12.6 (95% CI = 7.6 to 17.0) months compared with 14.3 (95% CI = 12.6 to 17.5) months for those with a negative lymph node 8a - log rank, p = 0.040 (*fig.16*).

fig.16 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients stratified by lymph node 8a status (n=140).



No. at risk						
LN8a -ve	109	59	27	11	6	4
LN8a +ve	31	16	2	0	0	0

Lymph node 16b status

Lymph node 16b was sampled in 87 cases and was found to be positive in 11 (12.6%). Patients with a positive lymph node 16b status had a median survival of 12.5 (95% CI = 7.5 to 27.4) months compared with 15.5 (95% CI = 12.3 to 22.1) months for those with a negative lymph node 16b - log rank, p = 0.261. *fig.17A* demonstrates the Kaplan-Meier survival curves for this analysis.

2.3.6. *Resection margin status*

Following histological microscopic re-assessment of all R0 resections, 163 evaluable cases were identified. Three cases were omitted due to an inability to reliably classify resection margin status from the available slides. Of the 128 cases (78.5%) classified as R1, 57 cases (44.5%) were based on tumour involvement within 1 mm of one or more margins, without direct involvement of the margin itself (ie. an 'equivocal' margin).

Table 7 demonstrates a breakdown of resection margin involvement according to the number of involved margins per specimen and to the distribution of margin involvement. These results indicate that 35.2% of R1 resections exhibit multifocal margin involvement (ie. more than one margin involved in a single specimen) while the posterior and medial margins were the most commonly involved margin locations.

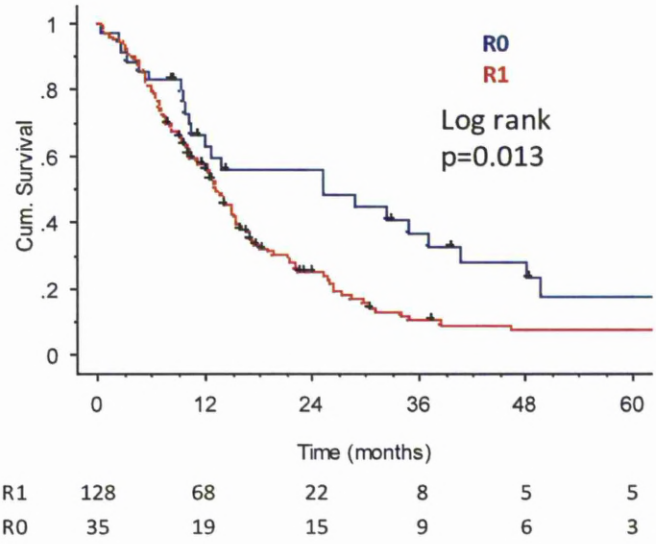
Table 7 - Distribution of resection margin involvement.

All R1 resections:	128 (79%)
Number of involved resection margins per specimen:	
1	83 (65%)
2	37 (29%)
3	7 (5%)
4	1 (1%)
Distribution of resection margin involvement:	
Posterior	69 (54%)
Medial	64 (50%)
Transection	38 (30%)
Proximal duodenal / gastric	6 (5%)
Common bile duct	4 (3%)
Distal duodenal	-

The prognostic relevance of 'equivocal' resection margins

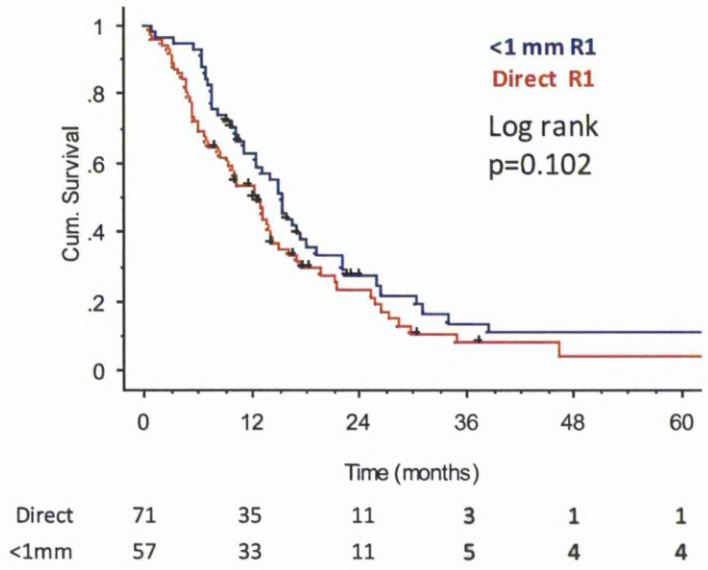
Equivocal R1 cases (<1mm) had a median survival of 15.4 (95% CI = 11.3 to 18.2) months compared with 12.6 (95% CI = 9.2 to 14.3) months for unequivocal R1 (direct) cases and 25.4 (95% CI = 10.5 to 40.8) months for R0 cases. When comparing the overall R1 group with R0 cases (*fig.18*), a significant difference in survival was recorded (log rank, p=0.013).

fig.18 - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients stratified by overall resection margin status (n=163).



When comparing equivocal with the unequivocal R1 cases (*fig.19*), no significant difference in survival was recorded (log rank, p=0.102). This was similarly true when comparing the equivocal R1 group with the R0 group (log rank, p=0.114). Despite this, the survival curves indicated much closer concordance between the equivocal and direct R1 cases than when comparing the equivocal and R0 groups. As such, equivocal cases were considered as R1 for the purposes of subsequent survival analyses.

fig.19 - Kaplan-Meier cumulative survival curves for R1 cases stratified by equivocal vs direct tumour involvement status (n=128).



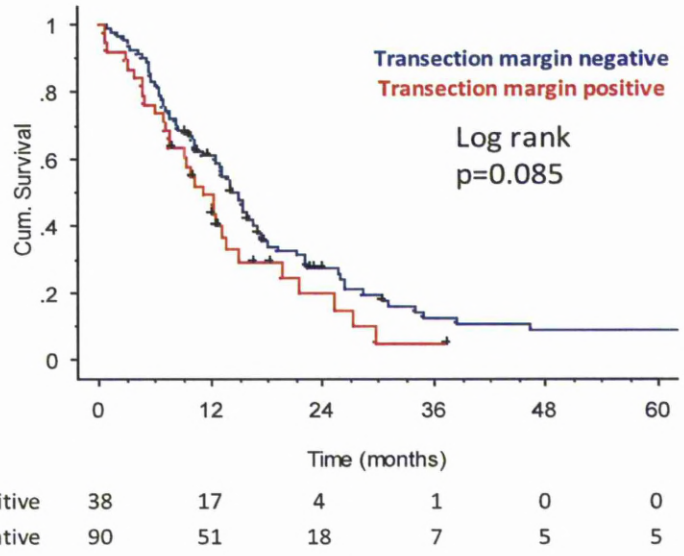
Prognostic relevance of margin location

When analysing only R1 resections, involvement of the transection margin was found to exhibit a non-significant trend towards poorer survival (log rank, p=0.085) - *fig.20*. *Table 10* demonstrates that neither posterior nor medial margin involvement conferred poorer survival within the R1 group.

Table 10 - Prognostic relevance of resection margin distribution in R1 cases (n=128).

Resection margin	**No. of patients**	**Median survival (95% CI)**	**p-value (log rank)**
Transection:			
Negative	90	15.0 (12.5 to 17.0)	0.085
Positive	38	11.3 (7.6 to 13.7)	
Posterior:			
Negative	59	13.3 (10.0 to 19.2)	0.259
Positive	69	13.8 (10.2 to 15.5)	
Medial:			
Negative	64	14.2 (8.5 to 15.5)	0.358
Positive	64	13.2 (11.1 to 17.4)	

fig.20 - Kaplan-Meier cumulative survival curves for R1 resections stratified by transection margin status (n=128).

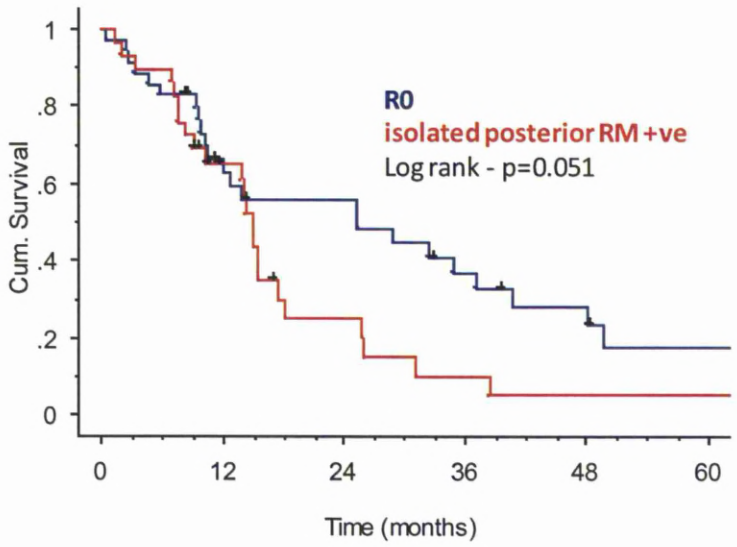


When analysing the group of 35 R0 resections, 12 cases exhibited isolated tumour involvement (either direct or <1 mm) of the anterior pancreatic capsule. There was no significant difference in survival between this group of patients and the remaining 23 R0 resections (log rank, p=0.220) - *fig.21A*. Due to the small numbers of cases involving duodenal and common bile duct resection margins, the prognostic relevance of these margins was not investigated.

Isolated involvement of posterior resection margin vs R0 cases

An analysis was undertaken to identify whether the survival of R1 cases with isolated posterior margin involvement (either <1mm or direct) was significantly different from R0 cases. A total of 29 R1 patients (22.7%) had isolated posterior margin involvement with a median survival of 15.0 (95% CI = 10.4 to 17.5) months compared with 25.4 (95% CI = 10.5 to 40.8) months for the 35 R0 cases - log rank; p = 0.051. *fig.22* demonstrates the Kaplan-Meier survival curves for this analysis.

fig.22 - Kaplan-Meier cumulative survival curves comparing survival between R1 cases with isolated posterior margin involvement and R0 cases.



No. at risk						
R0	35	19	15	9	6	3
Post. RM +ve	29	15	5	2	1	1

Prognostic relevance of number of involved resection margins

A further analysis was undertaken to identify whether an increasing number of involved resection margins within an individual pancreatoduodenectomy specimen conferred a poorer survival. Within the group of R1 resections (ie. both direct and equivocal), Cox regression failed to demonstrate any significant trend towards poorer survival in patients with an increasing number of involved resection margins - Cox; HR = 1.145 (95% CI = 0.914 to 1.436), χ^2 = 1.39, p = 0.238. *fig.23A* demonstrates that there was no significant survival difference when comparing R1 cases stratified according to single resection margin involvement vs. multiple margin involvement (log rank, p = 0.354).

Relationship between R1 status and other histological tumour characteristics

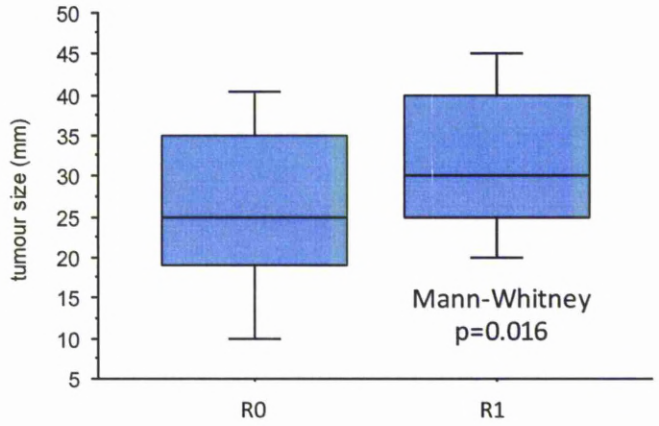
A logistic regression analysis was conducted to identify whether any of the other histological tumour characteristics were associated with an increased likelihood of microscopic margin involvement. Tumour size and T stage were included as continuous independent variables while tumour differentiation (poor vs. well/moderate) and nodal status (positive vs. negative) were included as categorical independent variables. R1 likelihood was included as the dependent variable in this analysis (*Table 11*).

Table 11 - Logisitic regression analysis to demonstrate relationship between tumour histology and likelihood of resection margin involvement.

	Logistic regression		
	Odds ratio (95% CI)	χ^2	p-value
Continuous:			
Size	1.049 (1.010 to 1.088)	6.205	**0.013**
T stage	1.644 (0.860 to 3.143)	2.266	0.132
Categorical:			
poor differentiation	2.164 (0.874 to 5.356)	2.786	0.095
+ve nodal status	1.187 (0.434 to 3.243)	0.112	0.738

Increasing tumour size (recorded in mm) was associated with a significantly increased likelihood of an R1 resection when included as a continuous independent variable (odds ratio = 1.049 (95% CI = 1.010 to 1.088); p=0.013). R0 resections had a median tumour size of 25 (IQR = 19 to 35) mm compared with 30 (IQR = 25 to 40) mm for R1 resections - *fig.24*. Poor tumour differentiation (p=0.095), increasing T stage (p=0.132) and nodal status (p=0.738) failed to exhibit a significant relationship with R1 likelihood in this patient cohort.

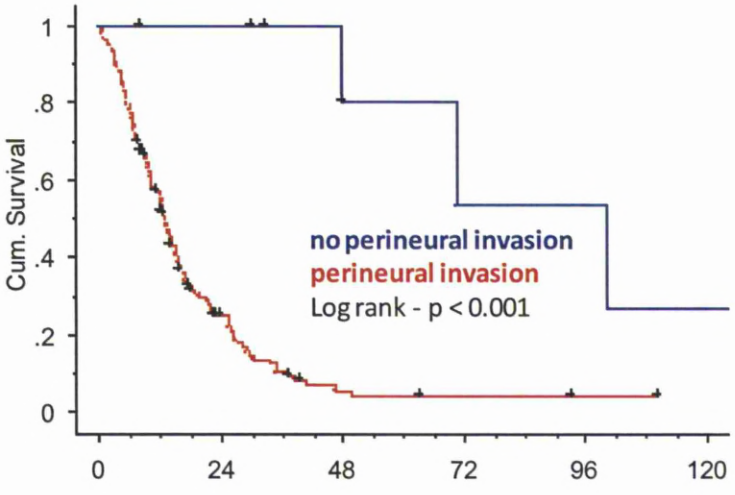
fig.24 - Box plot of tumour size according to resection margin status.



2.3.7. *Perineural invasion*

The presence or absence of perineural invasion on microscopic assessment was documented in 144 of 166 histology reports. Perineural invasion was present in 136 out of 144 cases (94.4%). Cases with perineural invasion had a median survival of 13.1 (95% CI = 10.5 to 15.0) months while cases with no perineural invasion had a median survival of 100.3 (95% CI = 48.1 to NR) months - log rank, $p<0.001$. The Kaplan-Meier survival curves for this analysis are demonstrated in *fig.25*.

fig.25 - Kaplan-Meier cumulative survival curves stratified according to the presence of perineural invasion (n=144).



No. at risk						
no peri. inv.	8	7	5	2	2	1
peri. invasion	136	25	4	2	1	0

Vascular invasion

The presence or absence of vascular invasion on microscopic examination was recorded in 138 of 166 cases. Vascular invasion was present in 104 out of 138 cases (75.4%). Cases with vascular invasion had a median survival of 13.1 (95% CI = 10.4 to 17.0) months while cases with no vascular invasion had a median survival of 15.0 (95% CI = 12.1 to 19.7) months - log rank, p=0.809. The Kaplan-Meier survival curves for this analysis are demonstrated in *fig.26A*.

Because of the small proportion of cases who failed to exhibit perineural invasion and the lack of any prognostic value of vascular invasion, neither of these histological factors were included in any of the subsequent multivariate analyses.

2.3.8. *Multivariate survival analysis of key univariate prognostic factors*

On the basis of the above analyses, the following histological variables of prognostic significance according to univariate analysis were considered for multivariate Cox proportional hazards regression. *Table 12* summarises the results of three multivariate analyses. The individual variables were modelled either on a continuous or dichotomised basis according to the principles outlined previously:

- Tumour size (continuous)
- Tumour differentiation (poor vs. well/moderate)
- Lymph node ratio (continuous)
- Resection margin status (R1 vs R0)

Table 12 - Multivariate survival analyses of standard histological prognostic factors alongside adjuvant chemotherapy in resected pancreatic ductal adenocarcinoma (Cox proportional hazards).

	Hazard ratio (95% CI)	χ^2	p
Model 1 (n=154)			
Tumour size	1.018 (1.002 to 1.035)	4.85	**0.028**
Poor tumour differentiation	1.430 (0.969 to 2.111)	3.24	0.072
Lymph node ratio	3.081 (1.266 to 7.499)	6.15	**0.013**
Resection margin +ve	1.436 (0.888 to 2.322)	2.18	0.140
Model 2 (n=154)			
Tumour size	1.017 (1.000 to 1.033)	4.03	**0.045**
Poor tumour differentiation	1.428 (0.966 to 2.110)	3.20	0.074
Lymph node ratio	2.984 (1.242 to 7.172)	5.97	**0.015**
Resection margin +ve	1.397 (0.861 to 2.267)	1.84	0.175
Adjuvant chemotherapy	0.598 (0.389 to 0.920)	5.48	**0.019**

A further analysis was conducted with exclusion of resection margin status as a covariate (*Table 13*).

Table 13 - Multivariate survival analysis of key prognostic factors in resected pancreatic ductal adenocarcinoma excluding resection margin status (Cox proportional hazards).

	Hazard ratio (95% CI)	χ^2	p
Model 3 (n=156)			
Tumour size	1.017 (1.002 to 1.034)	4.65	**0.031**
Poor tumour differentiation	1.551 (1.061 to 2.266)	5.14	**0.023**
Lymph node ratio	2.987 (1.238 to 7.183)	5.93	**0.015**
Adjuvant chemotherapy	0.580 (0.377 to 0.892)	6.15	**0.013**

2.3.9. *Discussion*

The multivariate analysis indicates that the four variables of increasing tumour size, poor differentiation, increasing lymph node ratio and lack of adjuvant chemotherapy are all independent adverse prognostic factors on multivariate analysis in this patient cohort. These key variables were selected for inclusion in all subsequent multivariate analyses undertaken.

Tumour size

Larger tumours were associated with significantly poorer survival outcomes in this cohort of pancreatic adenocarcinoma resections. This observation is typical of previous data from individual surgical centres (Garcea et al, 2008), adjuvant therapy trials (Neoptolemos et al, 2004) and meta-analyses (Stocken et al, 2005) of survival data from resected pancreatic cancer patients. Tumour size is commonly dichotomised according to a specific cut-off value (eg. > 20 mm) as part of these survival analyses which, as previously outlined, can be associated with potential bias. The results from the present data indicate that this relationship between increasing tumour size and adverse survival is equally true when analysing size as a continuous rather than dichotomised variable. Furthermore, tumour size demonstrated a stronger relationship with survival when analysed on a continuous basis. For this reason,

tumour size was modelled as a continuous covariate for subsequent multivariate analyses as this was felt to be the most informative method of describing this survival relationship.

Larger tumours were also demonstrated to exhibit a significant association with both the corresponding lymph node ratio and an increasing likelihood of a positive resection margin. Despite this fact, multivariate Cox regression demonstrated that the relationship between tumour size and survival was independently significant of these two factors. This observation suggests the presence of more extensive lymphangiogenesis and consequent regional nodal infiltration as the primary tumour size increases. Similarly, it follows that larger tumours occupying a greater proportion of the overall volume within the pancreatic head are more likely to exhibit microscopic tumour involvement at one or more resection margins as one might reasonably expect.

Differentiation

Tumour grade or differentiation represents an additional key histological tumour characteristic which has previously been shown to have a significant impact on survival following resection for pancreatic adenocarcinoma. Survival data from both ESPAC-1 and meta-analysed data including other adjuvant therapy trials (Stocken et al, 2005) have demonstrated that tumour differentiation is an important determinant of patient survival following resection. Due to the limited number of well differentiated tumours in the current patient cohort, along with the fact that there was no significant observed difference in survival between well and moderately differentiated tumours, these two tumour groups were combined for subsequent survival analyses. This was found to result in a significant survival difference when comparing the well / moderately differentiated tumour group with the poorly

differentiated tumour group. This categorisation was used for subsequent multivariate analyses.

Lymph node status

The UICC TNM classification for nodal status of resected pancreatic adenocarcinoma requires one of three categories be assigned:

- N0 - no tumour-involved lymph nodes present
- N1a - single tumour-involved lymph node present
- N1b - multiple tumour-involved lymph nodes present

Most studies investigating histological prognostic factors in resected pancreatic adenocarcinoma report nodal status as simply N0 or N1. The findings from the present study provide further evidence to suggest that this simple dichotomised categorisation results in restricted prognostic information. A previous large single-centre study (Pawlik et al, 2007) has shown that the number of tumour-involved lymph nodes as a proportion of the total number of sampled nodes in resected pancreatic cancer specimens provides superior prognostic information to overall nodal status (ie. positive vs. negative). This finding has also been observed from a recent analysis of SEER data (surveillance, epidemiology and end results) from a collective US database of 4005 pancreatic cancer resections (Slidell et al, 2008). Similar results have also been published for other gastrointestinal malignancies including colon (Berger et al, 2005) and gastric cancer (Inoue et al, 2002).

This observation is explained on the basis that patients with a smaller nodal tumour burden (eg. 1 of 20 sampled nodes) are more likely to exhibit longer survival times when compared to patients with a much greater overall nodal tumour burden (eg. 18 out of 20 sampled nodes)

who are consequently more likely to experience early regional recurrence. A comparative analysis of overall nodal status (ie. N1 vs. N0) against the lymph node ratio (modelled as a continuous variable) clearly shows that the latter demonstrates a much stronger association with patient survival. This allows additional risk stratification within the N1 group and provides significant supplementary prognostic information. For this reason, the lymph node ratio was included as a continuous covariate in subsequent multivariate analyses. This finding relating to the prognostic relevance of lymph node ratio within the current patient cohort was published in 2008 (*Appendix B*).

The analysis of lymph node yield indicated a significant relationship between the number of sampled nodes and the likelihood of an N1 classification and this observation has also been made previously (Slidell et al; 2008). The median number of nodes sampled in the overall patient group (17) compares favourably with other centres. Sliddell et al (2008) reported that in patients undergoing pancreatic resection for cancer a minimum of 12 sampled nodes represents the optimum harvest in order to reliably assess nodal status. The median number of nodes sampled from the 4005 resections included in their series of patients was seven. The number of sampled nodes in the present study was not found to exhibit any significant association with survival either in the overall patient group or the N0 subgroup. The previously reported observed relationship between reduced nodal harvest and poorer survival may not have been reflected in the current data by virtue of the fact that only a minority of cases (less than one in four) had fewer than 12 lymph nodes sampled. This represents an index of high quality pathology processing and reporting throughout the duration of the study period.

When analysing any potential relationships between the lymph node yield and corresponding histological tumour characteristics a significant association with T stage was recorded. The limited number of T1 and T2 tumours exhibited a smaller median number of sampled lymph nodes when compared with T3/T4 tumours. This observation is likely to be explained on the basis that patients with more extensive tumours underwent resections encompassing a greater amount of peripancreatic tissue in the specimen when compared with smaller tumours.

Resection margin status

The relative prognostic significance of resection margin status is variably reported for pancreatic cancer. Although several single centre studies have suggested that resection margin involvement has significant prognostic value on multivariate analysis alongside other histological tumour characteristics (Benassi et al, 2000; Han et al, 2006; Moon et al, 2006) studies including larger patient series typically demonstrate that R1 status either fails to maintain significance on multivariate analysis (Raut et al, 2007; Pawlik et al, 2007) or that R1 status fails to emerge as a significant univariate predictor of survival (Bassi et al, 2005; Jarufe et al, 2004). These results have also been mirrored in a previous meta-analysis of four adjuvant therapy trials which failed to demonstrate a significant overall survival difference according to resection margin status in a pooled group of 875 pancreatic adenocarcinoma resections (Butturini et al, 2008).

Highly variable R1 resection rates for pancreatic cancer are commonly quoted in different studies. Large multicentre adjuvant therapy trials have previously reported R1 resection rates of 17-19% (Neoptolemos et al, 2004; Oettle et al, 2007). However, these studies do not report potential differences in R1 rates between individual surgical centres. Studies reporting results from single centre cohorts demonstrate marked variability (17-85%) in R1 rates (Raut et al,

2007; Winter et al, 2006; Esposito et al, 2008; Verbeke et al, 2006). It is unknown as to what extent this heterogeneity in quoted R1 rates may be explained by differences in pathological practice rather than operative expertise. However, increasing evidence exists to suggest that the standard of histopathological processing and reporting has a significant impact on R1 resection rates (Esposito et al, 2008; Verbeke et al, 2006).

The previous study by Verbeke et al (2006) demonstrated that utilization of a standardised protocol for histological processing and examination of pancreatoduodenectomy specimens for pancreatic cancer based on the Royal College of Pathologists guidelines was associated with an R1 resection rate of 85%. This study also demonstrated a significant correlation between an increasing number of tissue blocks taken from circumferential margins and an increasing likelihood of an R1 classification. A more recent study by Esposito et al (2008) using a similar standardised histopathology protocol reported an R1 rate of 76%. These findings are consistent with the hypothesis that a negative resection margin status may be commonly incorrectly assigned to cases with sub-optimal pathological processing. The assertion that R1 resections are commonly under-reported is also supported by the observation that 60-80% of cases with resected pancreatic cancer develop local recurrence (Kayahara et al, 1993; Westerdahl et al, 1993; Sperti et al, 1997), a finding which seems incongruous with quoted R1 resection rates of less than 20%. Differences in histological R1 classification between individual centres may also in part explain the variable reporting of resection margin status as a prognostic index for pancreatic cancer.

The present study represents the first attempt to quantify the impact of the '<1 mm rule' in defining R1 classification for resected pancreatic cancer and provides further evidence to suggest that R1 resections may be commonly under-reported. The R1 resection rate of 79% in

this study group is comparable with the rates quoted by Esposito *et al* (76%) and Verbeke *et al* (85%) using standardised pathology protocols based on the Royal College of Pathologists guidelines. The results also suggest a similar proportion of multifocal R1 resections (35%) when compared with these two studies (32% and 45% respectively).

These findings indicate that the '<1 mm rule' has a significant impact on the quoted R1 resection rate. In total, 45% of all R1 resections in this cohort of patients were based on 'equivocal' margin involvement (ie. tumour within 1 mm of one or more margins in the absence of direct involvement). If these cases had been classified as R0, the R1 resection rate would fall significantly from 79% to 44%. Analysis of the survival curves supports the previous recommendation made in the Royal College of Pathologists' guidelines that tumour involvement within 1mm of a resection margin should be considered synonymous with incomplete excision. The group of 'equivocal' R1 resections exhibited a comparable survival distribution to the 'unequivocal' R1 group with no statistically significant difference when comparing the two survival curves. Further analysis of the survival data indicate that this classification system for R1 resections results in a significant prognostic index on univariate, but not multivariate, survival analysis.

When analysing the distribution of margin involvement in R1 resections for pancreatic cancer, the finding that posterior and medial margin involvement represent the most frequently involved margin locations is also consistent with the existing literature (Esposito et al, 2008; Verbeke et al, 2006; Nagakawa et al, 1996). When comparing survival within sub-groups of the R1 resections according to margin location, only cases with transection margin involvement demonstrated a trend (non-significant) towards poorer survival, indicating that

the transection margin represents the least favourable site for microscopic tumour involvement.

The analysis of the R0 resections indicates that the sub-group of patients with isolated tumour involvement of the anterior pancreatic capsule had no significant difference in survival when compared with the other R0 resections. Because of this observation, along with the fact that the anterior aspect of a pancreatoduodenectomy specimen represents a peritonealised surface as opposed to a true resection margin, the anterior 'margin' was not considered as a resection margin as part of this study. This issue represents an area where there is no clear pathological consensus in the literature (Esposito et al, 2008) and this finding requires validation in a larger cohort of patients to clarify the prognostic relevance of microscopic anterior tumour involvement.

Previous studies have suggested that the presence of poor tumour differentiation (Neoptolemos et al, 2001) and increasing tumour size (Raut et al, 2007) may be associated with an increased likelihood of resection margin involvement in pancreatic cancer. The results from the present study are consistent with regard to these findings. However, this observation only reached significance for tumour size. The association between these histological tumour characteristics and R1 likelihood may also in part explain why resection margin status commonly fails to emerge as a significant independent prognostic index when analysed in a multivariate context, as in the present study.

This analysis provides the first clinical evidence to validate the Royal College of Pathologists' guidelines regarding resection margin classification in pancreatoduodenectomy specimens for pancreatic cancer. The findings highlight the importance of standardised

histopathological reporting and provide a potential explanation for the significant heterogeneity in reported R1 resection rates quoted by different specialist cancer centres. The results also provide further evidence to suggest that histological tumour characteristics may be equally important determinants of R1 resections alongside robust pathological practice. These findings have been published (*Appendix B*) and have also been utilised as part of the criteria used to define resection margin status in pending revisions to the Royal College of Pathologists minimum dataset for pancreatoduodenectomy reporting (Campbell et al, 2002).

Additional histological tumour characteristics

Perineural invasion is a characteristic feature of pancreatic ductal adenocarcinoma and is invariably found on microscopic assessment of resected tumours. In the very small proportion of cases for who the pathologist failed to observe any perineural invasion, the observed survival times were more favourable. However, due to the limited number of these cases, perineural invasion did not represent an informative overall prognostic marker and was not included in any subsequent multivariate analyses. The presence of microscopic vascular invasion failed to exhibit any demonstrable impact on survival in the current patient cohort and this is a typical finding from previous large studies (Pawlik et al, 2007). Because no significant difference in overall survival was observed when comparing the pancreatic ductal adenocarcinoma cases stratified by T stage, along with the fact that a significant majority of cases exhibited T3 disease, this variable was not used in subsequent multivariate analyses when analysing other prognostic factors alongside established histological tumour characteristics.

With regard to the prognostic relevance of second order nodal disease, the results are in concordance with previous reports (Connor et al, 2004) which suggest that metastatic tumour

involvement of the common hepatic artery lymph nodes (8a) confers poorer survival when compared with those patients with negative lymph nodes sampled from this region. However, when excluding N0 cases from this analysis (ie. comparing survival according to LN8a status only within the N1 patient group) the result failed to reach significance ($p = 0.080$). Tumour involvement of the retroperitoneal nodes posterior to the head of the pancreas (LN16b) did not confer any additional prognostic information. As a result of these findings, along with the fact that a significant number of patients had neither second order nodes sampled as part of their surgery, these factors were not considered in subsequent multivariate analyses.

2.4. Preoperative prognostic factors

2.4.1. *Preoperative liver function and biliary drainage*

Table 14 outlines the distribution of biochemical results for 161 patients for whom preoperative liver function tests (LFTs) were available. 136 patients (84.5%) had preoperative LFTs recorded within 48 hours of surgery, 21 (13.0%) within 1 week and 4 (2.5%) within 2 weeks.

Table 14 - Details of preoperative liver function and biliary drainage procedures.

No. (%) of cases with preoperative LFTs recorded:	161 (97.0%)
Bilirubin: median (IQR)	25 (13 to 60) µmol/l
Alkaline phosphatase: median (IQR)	202 (123 to 362) U/l
ALT: median (IQR)	49 (25 to 84) U/l
γGT: median (IQR)	136 (64 to 360) U/l
Albumin: median (IQR)	36 (32 to 40) mg/l
Intervention for preoperative biliary drainage* (%):	
none	25 (15.1)
ERCP + stent	129 (77.7)
PTC / combined procedure + stent / drain	12 (7.2)
Interval from stenting to surgery: median (IQR)	34 (22 to 49) days

LFTs = liver function tests, ALT = alanine aminotransferase, γGT = gamma-glutamyl transferase, IQR = interquartile range, ERCP = endoscopic retrograde cholangio-pancreatography, PTC = percutaneous transhepatic cholangiopancreatography.

Four cases undergoing PTC required external drainage. A metal biliary stent was used in 5 out of 137 cases undergoing internal stenting. A plastic stent was employed in the remaining cases.

The effect of preoperative biliary drainage on survival

fig.27A demonstrates that there was no significant difference in early or overall survival when comparing those patients who did or did not undergo biliary drainage preoperatively (log rank, p=0.946). *fig.28A* demonstrates no significant difference in survival when comparing patients who required percutaneous intervention for biliary drainage with those who underwent endoscopic stenting (log rank, p=0.290).

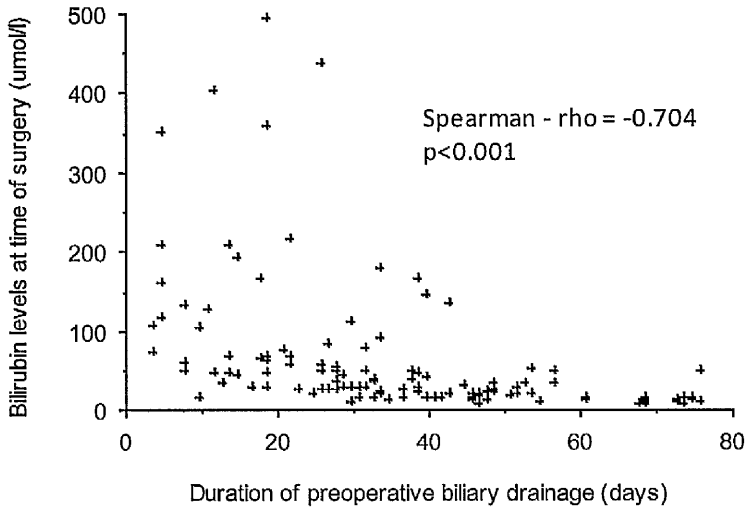
The effect of biliary drainage on resolution of jaundice

Patients requiring PTC exhibited a greater degree of residual jaundice at the time of surgery (median bilirubin level = 50 (IQR = 34 to 134) μmol/l) when compared with patients stented at ERCP (median bilirubin level = 24 (IQR = 13 to 47) μmol/l) - Mann-Whitney, p=0.042. There was a median reduction in bilirubin levels of 73% (IQR = 49% to 92%) in patients requiring PTC or combined procedures. However, only 3 of 12 patients (25%) experienced complete resolution of jaundice (ie. $\leq$ 35 μmol/l) at the time of surgery. In total, 64% (80/124) of patients undergoing stenting at ERCP had complete resolution of jaundice at the time of surgery.

Duration of preoperative biliary drainage and resolution of jaundice

When analysing the overall group of patients undergoing preoperative biliary drainage, there was a significant inverse correlation between the duration of biliary drainage and bilirubin levels prior to resection (Spearman, rho = -0.704, p<0.001) - ie. a longer period of preoperative biliary drainage resulted in lower bilirubin levels at the time of surgery (*fig.29*).

fig.29 - Plot to demonstrate inverse correlation between duration of biliary drainage and bilirubin levels



The influence of preoperative liver function on overall survival

Table 15 demonstrates the results of univariate Cox regression when modelling the various preoperative liver function parameters as continuous prognostic variables in the overall patient group undergoing resection for pancreatic cancer. The hazard ratios quoted for each variable reflect the increase in the relative hazard associated with each unit increase in the prognostic variable of interest.

Table 15 - Univariate Cox proportional hazards regression of preoperative liver function parameters.

	Hazard ratio (95% CI)	χ^2	**p**
Bilirubin	1.001 (0.999 to 1.003)	1.07	0.302
Albumin	0.958 (0.931 to 0.986)	8.53	**0.004**
Alkaline phosphatase	1.001 (1.000 to 1.001)	7.45	**0.006**
ALT	1.001 (0.998 to 1.004)	0.29	0.590
γGT	1.000 (1.000 to 1.001)	3.78	0.052

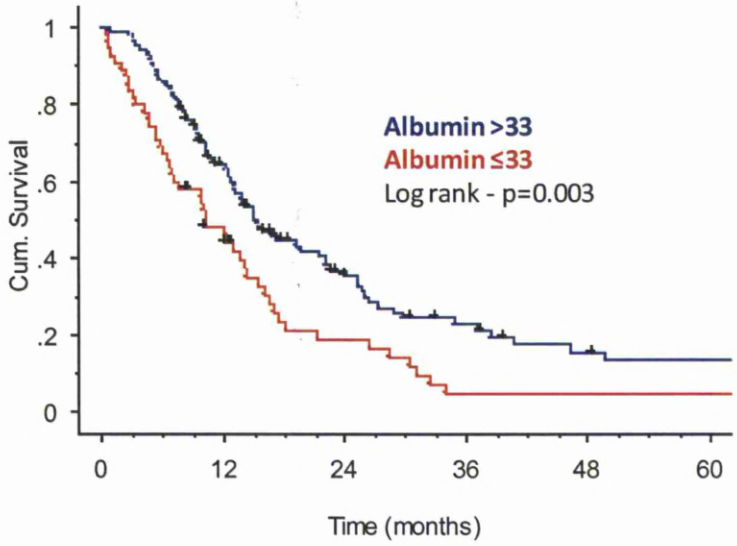
Univariate Cox analysis demonstrated a significant inverse association between preoperative albumin levels and postoperative survival (p=0.004) while elevated alkaline phosphatase levels were also associated with a significant trend towards poorer postoperative survival (p=0.006). Preoperative γ-glutamyl transferase levels were associated with a borderline significant result (p=0.052) while alanine aminotransferase exhibited no significant relationship with survival (p=0.590). *Table 16* demonstrates the results of a multivariate Cox analysis including albumin, alkaline phosphatase and γ-glutamyl transferase as covariates. This result demonstrates that of the three biochemical parameters, only preoperative albumin levels continue to exhibit a significant relationship with survival (p=0.039).

Table 16 - Multivariate Cox proportional hazards regression of preoperative liver function parameters.

	Hazard ratio (95% CI)	χ^2	**p**
Albumin	0.965 (0.933 to 0.998)	4.27	**0.039**
Alkaline phosphatase	1.000 (0.999 to 1.001)	0.30	0.583
γGT	1.000 (0.999 to 1.001)	0.46	0.497

fig.30 demonstrates the results of a Kaplan-Meier survival analysis dichotomising albumin according to a cut-off value of >33 g/l (ie. the lower limit of the normal reference range for serum albumin concentrations). Patients with a preoperative albumin >33 g/l had a median survival of 15.4 (95% CI = 13.1 to 22.1) months compared with 10.2 (95% CI = 6.9 to 14.3) months for patients with an albumin of ≤33 g/l (Cox; HR = 1.722 (95% CI = 1.202 to 2.468) - log rank, p = 0.003.

fig.30 - Kaplan-Meier cumulative survival curves according to preoperative hypoalbuminaemia.

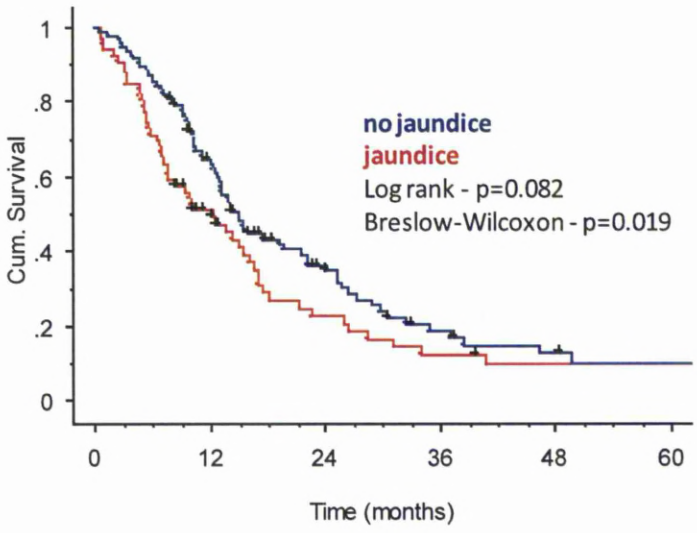


No. at risk						
Albumin >33	106	60	27	14	8	6
Albumin ≤33	55	24	8	2	2	2

The influence of preoperative jaundice on early postoperative survival

Although preoperative bilirubin levels were not found to exhibit a significant relationship with *overall* survival when modelled as a continuous variable, *fig.31* demonstrates a clear trend towards less favourable *early* survival in jaundiced patients (ie. bilirubin levels >35 μmol/l) at the time of resection (Breslow-Gehan-Wilcoxon, p=0.019). A cut-off of >35 μmol/l was selected as this represents the serum concentration at which hyperbilirubinaemia is clinically apparent as jaundice.

fig.31 - Kaplan-Meier cumulative survival curves according to preoperative jaundice.



No. at risk						
no jaundice	95	56	24	10	6	4
jaundice	66	28	11	6	4	4

Table 17 demonstrates the results of a multivariate Cox analysis including serum albumin as a continuous covariate alongside tumour size, differentiation, lymph node ratio and adjuvant chemotherapy (n=152). The results indicate that low preoperative serum albumin levels continue to exhibit an adverse relationship with overall survival (p = 0.002) alongside the other prognostic factors of relevance.

Table 17 - Multivariate Cox analysis of preoperative albumin alongside tumour histology / chemo.

(n=152)	**Hazard ratio (95% CI)**	χ^2	p
Albumin	0.952 (0.923 to 0.983)	9.32	**0.002**
Tumour size	1.019 (1.003 to 1.036)	5.34	**0.021**
Poor differentiation	1.598 (1.090 to 2.344)	5.76	**0.016**
Lymph node ratio	4.415 (1.717 to 11.354)	9.49	**0.002**
Adjuvant chemotherapy	0.612 (0.391 to 0.959)	4.60	**0.032**

Discussion

There is currently no general consensus regarding whether preoperative biliary drainage prior to surgical intervention represents the optimal management approach in patients presenting with potentially resectable pancreatic cancer. A meta-analysis has suggested that preoperative intervention for biliary drainage is associated with an increased risk of early postoperative morbidity, principally relating to wound infection (Sewnath et al, 2002). However, no overall association between biliary drainage and perioperative mortality was identified in this study. This meta-analysis was based on level 1 evidence from five randomised trials comprising 302 periampullary cancers in total. Less than half of these patients had pancreatic adenocarcinoma and a similar proportion of the overall group actually underwent resection. These five trials encompassed a mix of both endoscopic and percutaneous procedures and included cases undergoing both internal and external drainage. The median bilirubin level at the time of surgery for the pooled patient group undergoing preoperative biliary drainage was recorded as 157 μmol/l. This represents a significantly greater value than the result recorded in the present study (25 μmol/l). Given the mix of periampullary tumours and the inclusion of both resected and unresected cases in the above meta-analysis, the overall findings from this study might not be reliably extrapolated to the specific setting of resected pancreatic cancer. Nevertheless, the results of the present study are concordant with the above findings in that biliary stenting *per se* was not shown to have any adverse effect on early or late survival in the overall patient group undergoing pancreatoduodenectomy for pancreatic cancer.

One of the main observations from the above analysis was the association between residual jaundice at the time of surgery and less favorable early survival. A previous study has also suggested that preoperative jaundice may represent a significant predictor of postoperative survival in a cohort of 281 resected periampullary cancers (Schmidt et al, 2004). In the

present study, separation of the survival curves was evident primarily for the first 12 postoperative months resulting in a significant p-value when using the Breslow-Gehan-Wilcoxon test, but not the log rank (Cox-Mantel) test. The Breslow-Gehan-Wilcoxon test is calculated according to the number of patients at risk along each point of the survival curve and provides a better discriminator of differences in early survival between two groups - ie. the follow-up period during which the majority of patients are still alive. In comparison, the log rank test, which is calculated according to equal weighting at each point along the survival curve, provides a better indication of differences in overall and late survival.

The results demonstrate that the majority of patients with resectable pancreatic cancer who present with obstructive jaundice can undergo successful preoperative biliary drainage at ERCP. The findings also demonstrate that a longer period of preoperative biliary drainage was inversely correlated with bilirubin levels at the time of surgery as one might expect. This suggests that a balance exists with regard to the optimal timing of definitive surgery for this patient group, in order to allow for resolution of jaundice where possible without compromising the window of opportunity for tumour resectability. This issue is particularly relevant for patients where borderline features of resectability are present on initial imaging or where resolution of jaundice is protracted, even following percutaneous intervention. The decision-making process regarding the optimal timing of surgery is clearly a multifactorial one which may need to incorporate a number of additional logistical issues and should be considered on an individual patient basis. However, the observations from the present study indicate that early survival outcomes may be adversely influenced by inadequate resolution of preoperative jaundice.

These findings were published in 2008 (*Appendix B*). The recently published multicentre DROP-trial randomised 202 jaundiced patients with pancreatic head and peri-ampullary cancers to undergo either preoperative biliary drainage prior to resection or early surgery (van der Gaag et al. 2010). The results indicated a greater degree of perioperative sepsis-related morbidity in the patient group undergoing biliary drainage. However, a further sub-group analysis of this data (Eshuis et al. 2010) found that there was no difference in resectability rates or overall postoperative survival when comparing the stented patients with those undergoing early surgery. Despite these results, they also observed a significant trend towards a *lower* operative mortality rate in patients with a longer time interval between randomisation and surgery for the overall study group. This observation is in agreement with our finding that patients with complete resolution of jaundice following biliary stenting had improved early survival when compared with those who remained jaundiced at the time of their operation. This suggests that the issue of perioperative morbidity is only one of a number of factors to consider when evaluating whether preoperative biliary drainage or expeditious resection represents the optimum management strategy for these patients.

Preoperative albumin levels were found to be a significant predictor of overall survival in the present study. Although previous studies have demonstrated that hypoalbuminaemia is associated with less favourable survival outcomes in patients with inoperable pancreatic cancer (Glen et al, 2006) and higher morbidity and mortality rates following pancreatoduodenectomy (Winter et al, 2007), no previous study has shown that pre-resection albumin levels are also associated with overall postoperative survival following resection for pancreatic cancer (Jamieson et al, 2005). Hypoalbuminaemia is often associated with a systemic inflammatory response and the observed survival association may simply reflect the fact that preoperative albumin levels represent an additional index of inflammation.

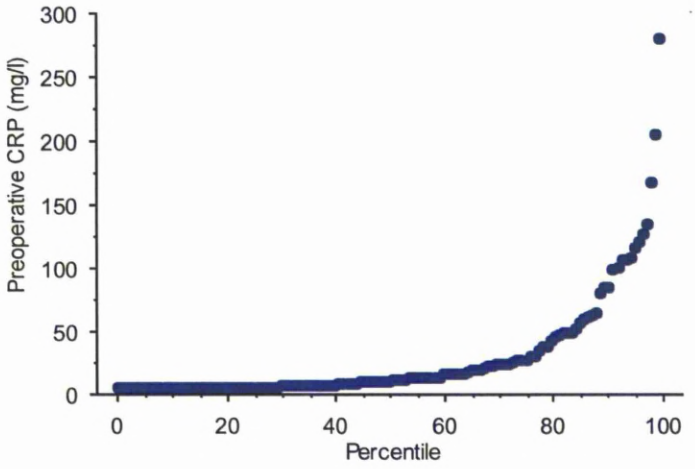
Alternatively, low albumin concentrations may represent a multifactorial surrogate marker of general ill-health prior to surgery (ie. poorer overall nutritional status, impaired liver function, cancer cachexia, etc) which adversely affects survival. This finding raises the possibility that preoperative measures to counteract hypoalbuminaemia may influence postoperative outcome. A recent study has suggested that the introduction of immuno-enriched nutritional supplements in the preoperative setting yields significant improvements in early postoperative outcomes for patients undergoing major pancreatic resections (Giger et al, 2007).

2.4.2. *Preoperative serum C-reactive protein (CRP)*

Preoperative serum CRP levels were recorded in 131 patients (81.4%). The median time interval from the date of CRP estimation to date of surgery was 2 (IQR = 1 to 12 days). The median preoperative CRP recorded was 10 mg/l (IQR = 5 to 27). Where a CRP level was recorded as <5 mg/l, a value of 4 mg/l was used for subsequent analyses.

fig.32 demonstrates a percentile plot of the distribution of preoperative CRP levels. This suggests that over 90% of cases had CRP levels less than 100 mg/l with 10 cases in whom levels of >100 mg/l were recorded.

fig.32 - Percentile plot of preoperative CRP results recorded (n=131).



A univariate Cox proportional hazards regression analysis was conducted using preoperative CRP as a continuous prognostic covariate. *Table 18* demonstrates the results of this analysis when including all patients for whom CRP levels were recorded and when excluding the 10 cases for whom levels of >100 mg/l were recorded.

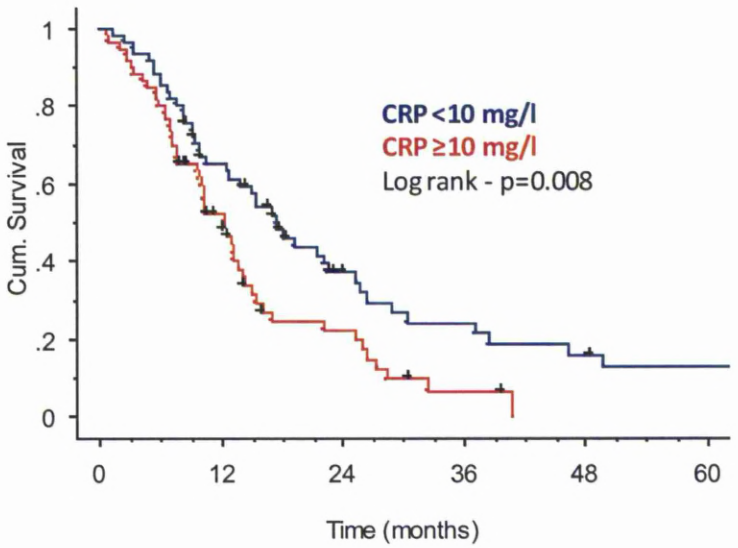
Table 18 - Univariate Cox analysis of preoperative CRP levels as prognostic index (continuous).

	Hazard ratio (95% CI)	χ^2	p
All patients	0.998 (0.994 to 1.002)	1.14	0.287
Excluding cases with CRP >100	1.011 (1.001 to 1.020)	5.12	**0.024**

This result suggests that there is a significant underlying relationship between preoperative CRP levels and subsequent survival following resection for pancreatic ductal adenocarcinoma on a univariate basis. However, it also implies that the presence of CRP levels >100 mg/l (which is more likely to reflect concurrent infective complications (eg. cholangitis) rather than the host inflammatory response to tumour) has a marked confounding effect when analysing the prognostic value of CRP levels in this setting.

fig.33 demonstrates the Kaplan-Meier cumulative survival curves when stratifying patients according to a preoperative CRP of >10 mg/l (excluding patients with levels >100 mg/l). A cut-off value of 10 mg/l was selected on the basis that this value is commonly used to define the upper limit of normal for serum CRP in a clinical setting.

fig.33 - Kaplan-Meier cumulative survival curves according to preoperative CRP levels.



No. at risk						
CRP <10 mg/l	61	36	15	9	6	4
CRP ≥10 mg/l	60	27	9	2	0	0

Patients with a preoperative CRP <10 mg/l had a median survival of 17.0 (95% CI = 12.8 to 22.1) months compared with 12.3 (95% CI = 9.8 to 13.7) months for patients with a CRP of ≥10 mg/l (log rank, p = 0.008). *Table 19* outlines the results of a multivariate Cox proportional hazards analysis including preoperative CRP levels (included as a continuous covariate) alongside the histological prognostic factors of relevance.

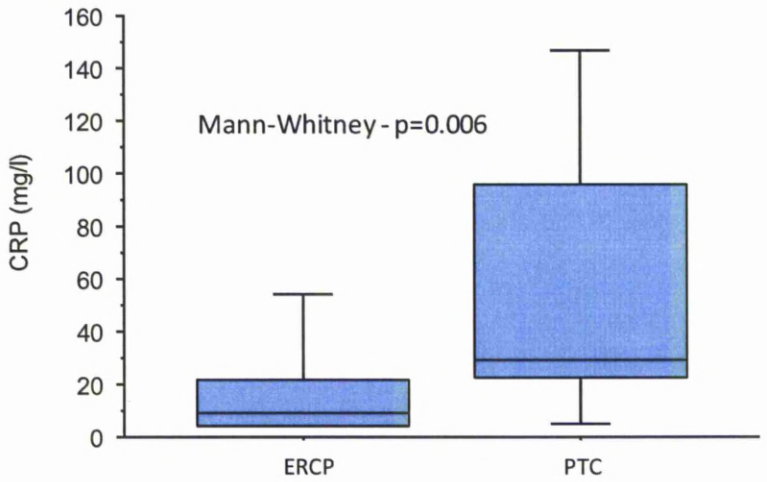
Table 19 - Multivariate Cox analysis of preoperative CRP alongside tumour histology / chemo.

(n=114)	**Hazard ratio (95% CI)**	χ^2	**p**
CRP	1.012 (1.002 to 1.023)	5.19	**0.023**
Tumour size	1.014 (0.994 to 1.033)	1.86	0.173
Poor differentiation	1.358 (0.866 to 2.127)	1.78	0.182
Lymph node ratio	5.477 (1.656 to 18.116)	7.77	**0.005**
Adjuvant chemotherapy	0.603 (0.370 to 0.981)	4.15	**0.042**

The relationship between preoperative biliary drainage and CRP

Preoperative CRP levels were found to be more significantly elevated in those cases requiring PTC (median (IQR) = 29 (22 to 107) mg/l) when compared with those stented at ERCP (median (IQR) = 9 (4 to 21) mg/l); Mann-Whitney, p=0.006 (*fig.34*).

fig.34 - Box plot of preoperative CRP levels according to type of biliary drainage.



Discussion

Elevated CRP levels were found to be associated with adverse survival when excluding patients with preoperative CRP values >100 mg/l. Cholangitis represents the most common cause for acute sepsis preoperatively in patients undergoing biliary decompression and stenting has also been shown to be associated with positive bile cultures at laparotomy (Jagannath et al. 2005; Jethwa et al. 2007). Several studies have demonstrated that pre-resection CRP levels represent a potential prognostic factor in other gastrointestinal malignancies (Nozoe et al. 2001; Hashimoto et al. 2005; Crozier et al. 2007). In the single previous study in resected pancreatic cancer (n=65), an adverse association between elevated preoperative CRP and survival was shown on univariate but not multivariate analysis (Jamieson et al. 2005).

The results from the present study demonstrate that the relationship between elevated preoperative CRP levels and poorer survival is only evident when excluding a small number of outlying patients with a significantly elevated CRP. It was not possible to determine which patients in this study had preoperative clinical features of cholangitis on a retrospective basis and the selection of a CRP cut-off value of 100 mg/l for exclusion was a pragmatic one rather than being based on any pre-defined diagnostic criteria. However, when analysing the 10 excluded patients with CRP levels >100 mg/l, 9 underwent preoperative intervention for biliary drainage and the median concurrent bilirubin level at the time of CRP estimation was 92 (IQR = 33 to 160) μmol/l. This observation is concordant with the only other study which has investigated the relationship between preoperative CRP levels and postoperative survival in resected pancreatic cancer (Jamieson et al, 2005). This study also reported that patients with cholangitis were excluded from their analysis. However, they failed to elaborate on any specific criteria with regard to how this was defined. It was, therefore, felt to be reasonable to conclude that the disparity in the survival analyses outlined above was principally due to the confounding effect of cholangitis in the group of patients with significantly elevated CRP levels. Tumour size and differentiation lost significance with inclusion of CRP in the multivariate analysis. This is likely to reflect a type II error as a result of the smaller number of patients included within this model and this finding is similarly true for the subsequent analysis of CA19-9.

Preoperative percutaneous intervention for biliary drainage was associated with higher CRP levels at the time of surgery. Percutaneous access to the biliary tree is a more invasive route than that associated with ERCP and patients who required PTC or combined procedures would have already undergone one or more unsuccessful attempts at endoscopic biliary stenting. Therefore, the association between elevated CRP levels and PTC is likely to reflect

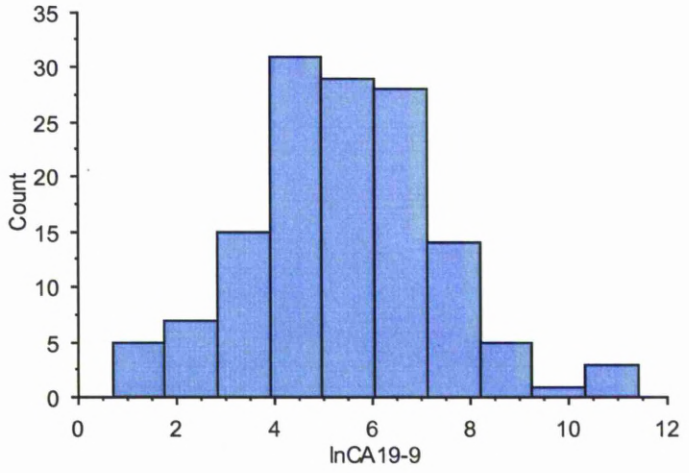
the increased tissue damage associated with percutaneous access in addition to increasing the degree of bactobilia and consequent likelihood of biliary sepsis due to the cumulative effect of previous endoscopic biliary instrumentation prior to successful drainage. The results suggest that while elevated preoperative CRP levels exhibit an association with overall survival, the presence of cholangitis and the requirement for percutaneous biliary decompression are likely to represent significant confounding factors when interpreting the prognostic value of preoperative CRP levels in this patient group. These findings were published in 2008 (*Appendix B*).

2.4.3. *Preoperative serum CA19-9*

Preoperative serum CA19-9 levels were recorded for 138 patients. In cases where more than one preoperative value was recorded, the result taken nearest to the date of surgery was used for analysis. The median preoperative CA19-9 level was 236 (IQR = 68 to 681) kU/l. The median time interval from the date of preoperative CA19-9 estimation to surgery was 26 (IQR = 17 to 36) days. 19 of the 138 patients did not undergo preoperative biliary drainage. In the remaining group, CA19-9 levels were recorded prior to stenting in 46 (38.7%) and following stenting in 73 (61.3%).

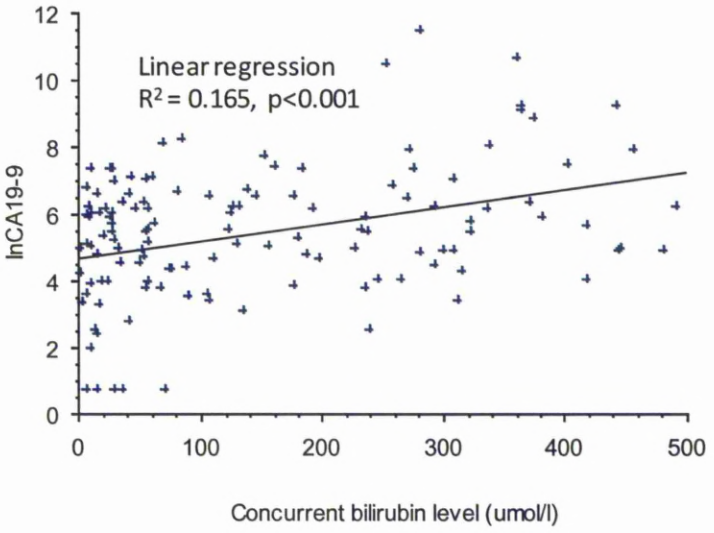
Due to the wide range of CA19-9 results recorded (from 2 kU/l to 90,000 kU/l), the natural logarithm was used for subsequent regression and correlation analyses. *fig.35* demonstrates that the preoperative CA19-9 results were approximately normally distributed when calculating the natural logarithm (ie. lnCA19-9).

fig.35 - Distribution of preoperative CA19-9 results normalised by logarithmic transformation.



A significant association between preoperative lnCA19-9 and concurrent bilirubin levels was demonstrated in 131 cases where both results were available - *fig.36*.

fig.36 - Scatterplot to demonstrate relationship between preoperative lnCA19-9 and bilirubin levels.



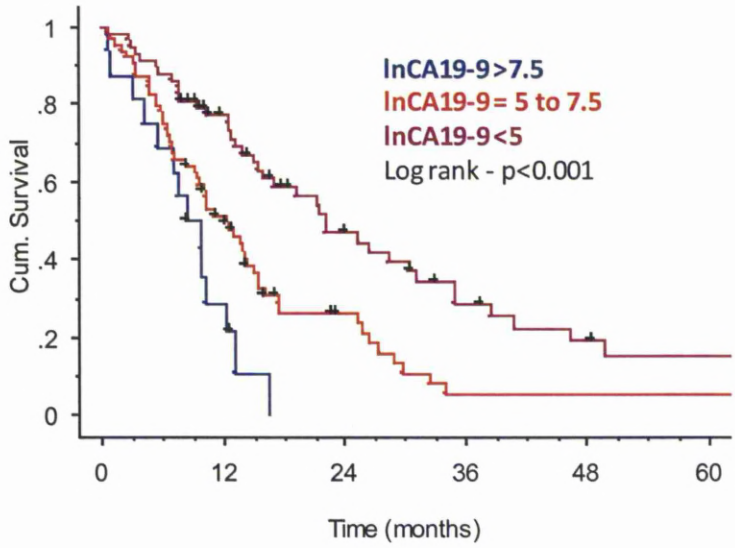
CA19-9 and univariate survival

Preoperative CA19-9 exhibited a significant relationship with overall survival when analysed as a continuous prognostic variable. *Table 20* outlines the results of univariate Cox proportional hazards regression. The strong association between lnCA19-9 and survival allowed three discrete risk groups to be defined. *fig.37* demonstrates the Kaplan-Meier cumulative survival curves for lnCA19-9 according to these three categories (ie. <5, 5 to 7.5, >7.5).

Table 20 - Univariate survival analysis of preoperative lnCA19-9 as a continuous and categorical prognostic variable (n=138).

	Median survival (95% CI) months	**Hazard ratio (95% CI)**	χ^2	**p-value**
lnCA19-9 (3groups):		-	21.56	**<0.001**
<5	22.1 (15.4 to 31.2)	-	-	-
5 to 7.5	12.3 (9.5 to 15.0)	2.065 (1.352 to 3.155)	11.25	**<0.001**
>7.5	8.5 (5.5 to 12.4)	4.227 (2.202 to 8.115)	18.77	**<0.001**
lnCA19-9:				
continuous	-	1.245 (1.119 to 1.385)	16.13	**<0.001**

fig.37 - Kaplan-Meier cumulative survival curves according to three preoperative lnCA19-9 groups.



No. at risk						
>7.5	16	4	0	0	0	0
5 to 7.5	64	30	10	2	2	2
<5	58	38	20	10	6	4

CA19-9 and multivariate survival

Table 21 outlines the results of a multivariate Cox regression analysis including preoperative lnCA19-9 as a continuous prognostic variable alongside the histological prognostic factors of relevance. The results indicate that elevated preoperative CA19-9 levels continue to exhibit a strong adverse relationship with survival (p=0.005).

Table 21 - Multivariate Cox analysis of preoperative lnCA19-9 alongside tumour histology / chemo.

(n=130)	**Hazard ratio (95% CI)**	χ^2	p
lnCA19-9	1.185 (1.052 to 1.336)	7.74	**0.005**
Tumour size	1.014 (0.996 to 1.031)	2.43	0.119
Poor differentiation	1.532 (0.999 to 2.350)	3.83	0.050
Lymph node ratio	3.747 (1.182 to 11.878)	5.04	**0.025**
Adjuvant chemotherapy	0.565 (0.346 to 0.923)	5.20	**0.023**

Relationship between CA19-9 and tumour histology

Table 22 outlines the association between preoperative CA19-9 and associated tumour histology. The results indicate that CA19-9 levels are generally greater in patients with larger tumours and those patients with lymph node involvement. Spearman's rank correlation also indicated a significant association between increasing tumour size and elevated lnCA19-9 (rho = 0.204, p = 0.022). There was no significant correlation between the lymph node ratio and lnCA19-9 (Spearman, rho = 0.075, p=0.400).

Table 22 - Relationship between preoperative CA19-9 and histological tumour characteristics.

	Median CA19-9 level kU/l (IQR)	p-value*
Tumour Size:		
≤20mm	122 (35 to 324)	**0.010**
>20mm	356 (90 to 859)	
Nodal Status:		
negative	128 (30 to 384)	0.056
positive	280 (85 to 750)	
Resection margin:		
negative	136 (55 to 492)	0.325
positive	236 (73 to 785)	
Differentiation:		
well / moderate	172 (52 to 630)	0.099
poor	315 (134 to 860)	

p-values for Mann-Whitney test

Discussion

The results from the present study confirm that greater preoperative CA19-9 levels are associated with a significantly reduced overall survival following resection for pancreatic ductal adenocarcinoma. Normalisation of CA19-9 levels following resection for pancreatic cancer has been shown to be associated with more favourable subsequent survival in patients undergoing resection for pancreatic cancer (Sperti et al, 1993; Montgomery et al, 1997; Safi et al, 1998). However, only a small number of studies have investigated the potential value of preoperative CA19-9 levels in isolation as a prognostic index (Lundin et al, 1994; Kau et al, 1999; Ferrone et al, 2006). These studies characteristically dichotomise the number of patients into high- and low-risk groups according to a single cut-off value for CA19-9 without any attempt to correct for the potential bias associated with this approach. The results from the present study indicate that CA19-9 exhibits a strong relationship with survival both

as a continuous and categorical prognostic variable, thereby avoiding any potential bias associated with categorising continuous prognostic data (Altman et al, 1994).

Preoperative serum CA19-9 levels demonstrated a significant association with concurrent bilirubin levels in the present patient cohort. CA19-9 is secreted in a mucin-bound form by the biliary and gallbladder mucosa and excreted in bile (Ker et al, 1991; von Ritter et al, 1997; Brockmann et al, 2000) and existing studies have demonstrated that obstructive jaundice will commonly precipitate elevated serum concentrations in the absence of malignancy (Duraker et al, 2007). Given the previous findings indicating that preoperative bilirubin levels had no significant effect on overall survival when analysed on a continuous basis, there was no evidence to indicate that concurrent jaundice exerted any significant confounding effect to explain the strong association between preoperative CA19-9 and survival. These results are also consistent with the findings from a previous study (Kim et al, 1999) which demonstrated that, in the context of diagnosing pancreatic malignancy in symptomatic patients, the presence of concurrent obstructive jaundice did not significantly affect the sensitivity or specificity of CA19-9 when using a standard diagnostic cut-off value of 37 kU/l.

The deleterious survival outcome observed for cases with elevated preoperative CA19-9 levels is likely to be explained on the basis that elevated CA19-9 was observed for larger tumours and cases exhibiting nodal involvement. There was also a non-significant trend towards elevated CA19-9 in patients with poorly differentiated tumours. These findings support the assertion that preoperative CA19-9 levels are not only indicative of tumour burden, but also that CA19-9 may act as a marker of biological 'aggressiveness'. This hypothesis is borne out by the finding that preoperative CA19-9 was a more significant

variable when compared to tumour size or nodal status alone. Given that the presence of local or distant micrometastates at the time of surgery is believed to be the most significant factor in limiting long-term survival for the majority of resected pancreatic cancer patients (Bogoevski et al, 2004), it is reasonable to hypothesise that preoperative CA19-9 levels may also act as a marker of disseminated micrometastatic disease. These findings have been published in 2008 (*Appendix B*).

2.4.4. *Platelet-lymphocyte ratio*

A complete preoperative full blood count (FBC) was available in 147 (88.6%) evaluable cases and the results taken nearest to the date of surgery were used for analysis. The median time interval from the date of preoperative FBC to date of surgery was 1 (IQR = 1 to 2) days.

fig.38 demonstrates the distribution of preoperative lymphocyte counts. The median lymphocyte count was 1.9 (IQR = 1.3 to 2.4) $x10^9$/l. Lymphocytopaenia (ie. a lymphocyte count less than 1.0 $x10^9$/l) was present in 10 patients (6.8%).

fig.38 - Distribution of preoperative lymphocyte counts.

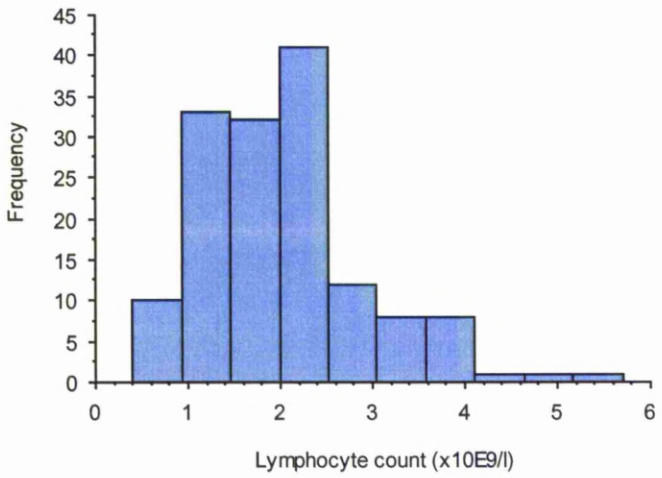


fig.39 demonstrates the distribution of preoperative platelet counts. The median recorded platelet count was 323 (IQR = 265 to 404) $x10^9$/l. Thrombocytosis (ie. platelet count >400 $x10^9$/l) was present in 40 patients (27.2%).

fig.39 - Distribution of preoperative platelet counts.

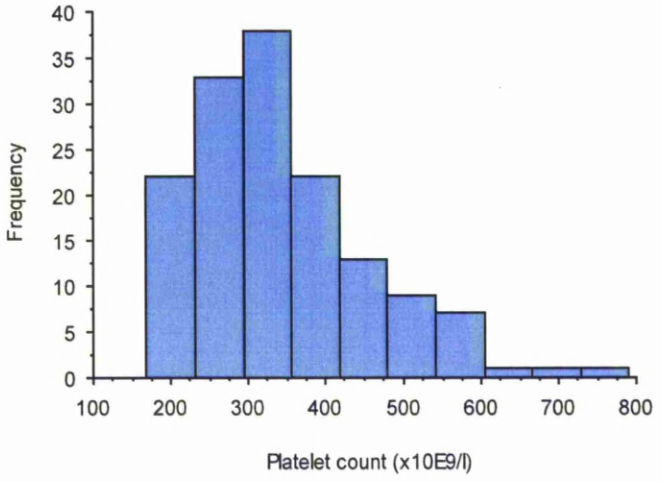
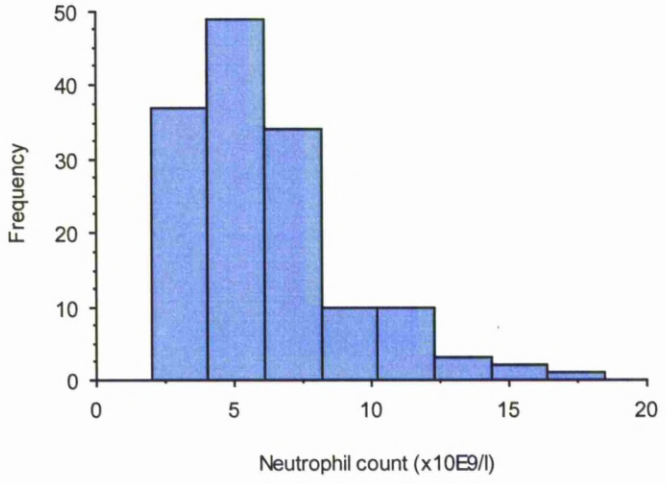


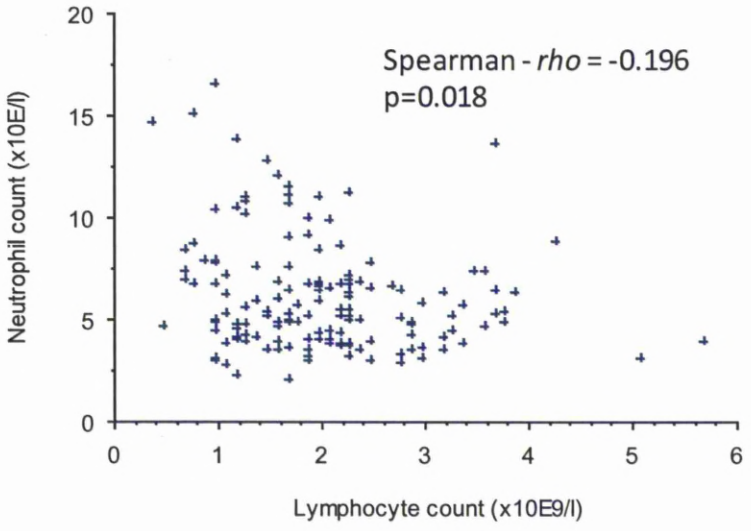
fig.40 demonstrates the distribution of preoperative neutrophil counts. The median recorded neutrophil count was 5.3 (IQR = 4.1 to 7.1) $x10^9/l$. Neutrophilia (ie. neutrophil count >7.0 $x10^9/l$) was present in 38 patients (25.9%).

fig.40 - Distribution of preoperative neutrophil counts.



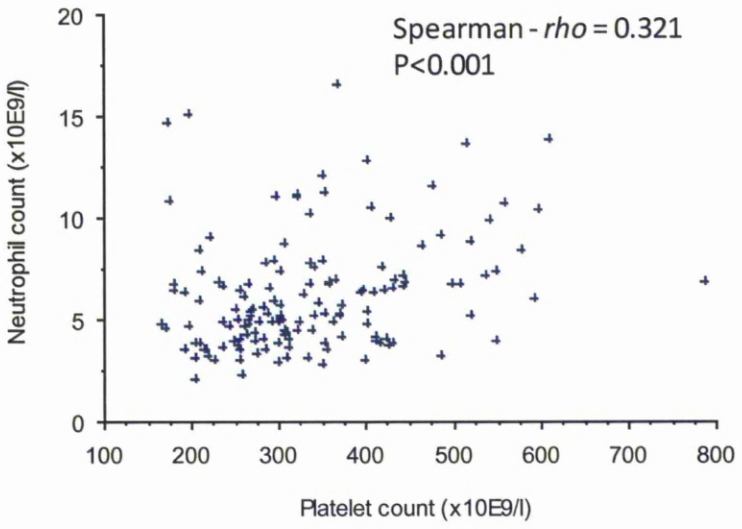
There was a significant inverse relationship between the preoperative neutrophil and lymphocyte counts (Spearman, *rho* = -0.196 (95% CI = -0.347 to 0.035), p = 0.018) - *fig.41*. This result demonstrates the expected trend towards lower lymphocyte counts in patients exhibiting greater neutrophil counts indicating that a relative lymphocytopaenia represents an additional index of the host inflammatory response to tumour.

fig.41 - Scatterplot to demonstrate inverse correlation between preoperative lymphocyte and neutrophil counts.



A significant association between increasing platelet and neutrophil counts was also observed (Spearman, *rho* = 0.321 (95% CI = 0.168 to 0.459), p < 0.001) - *fig.42*. Similarly, this observation suggests that the platelet count reflects a further potential marker of systemic inflammation in this patient group.

fig.42 - Correlation between preoperative platelet and neutrophil counts.



Univariate survival according to preoperative haematological parameters

The results of univariate survival analysis using Cox proportional hazards regression for each of the haematological parameters (ie. lymphocyte, neutrophil and platelet counts) are shown in *Table 19*.

Table 23 - Univariate survival analysis of preoperative haematological parameters as continuous prognostic covariates (Cox proportional hazards).

	Hazard ratio (95% CI)	**Chi-square**	**p-value**
Lymphocyte count ($x10^9$/l)	0.819 (0.665 to 1.009)	3.51	0.061
Neutrophil count ($x10^9$/l)	1.047 (0.988 to 1.110)	2.42	0.120
Platelet count ($x10^9$/l)	1.002 (1.001 to 1.004)	7.56	**0.006**
N/L ratio	1.019 (0.977 to 1.062)	0.78	0.377
P/L ratio	1.003 (1.001 to 1.004)	10.81	**0.001**

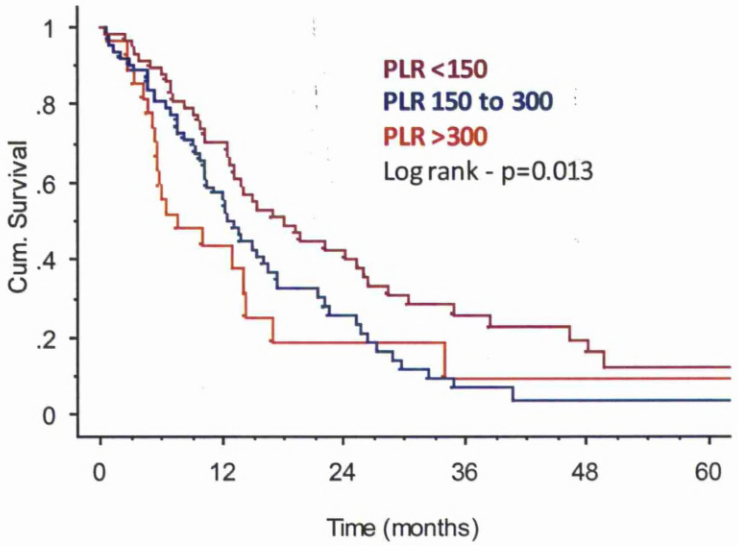
N/L ratio = neutrophil-lymphocyte ratio, P/L ratio = platelet-lymphocyte ratio

Lower lymphocyte counts exhibited a borderline significant relationship with poorer survival (p=0.061) while increasing platelet counts were significantly associated with adverse survival (p=0.006). The platelet-lymphocyte ratio (PLR) was demonstrated to be a superior prognostic index when compared with either parameter in isolation (p=0.001). Neither the neutrophil count nor the neutrophil-lymphocyte ratio (NLR) was demonstrated to confer significant prognostic information in this patient cohort. *Table 24* outlines the survival of three patient sub-groups stratified according to the preoperative PLR. The corresponding Kaplan-Meier cumulative survival curves for these three patient groups are shown in *fig.43*.

Table 24 - Univariate survival analysis of preoperative platelet-lymphocyte ratio as a continuous and categorical prognostic variable (n=147).

	Median survival (95% CI) months	**Hazard ratio (95% CI)**	χ^2	**p-value**
PLR (3groups):		-	8.48	**0.014**
<150	18.2 (13.3 to 26.5)	-	-	-
150 to 300	13.2 (10.4 to 16.6)	1.626 (1.077 to 2.455)	5.35	**0.021**
>300	7.6 (5.5 to 14.3)	2.025 (1.188 to 3.451)	6.73	**0.010**
PLR:				
continuous	-	1.003 (1.001 to 1.004)	10.81	**0.001**

fig.43 - Kaplan-Meier cumulative survival curves according to three platelet-lymphocyte ratio groups.



No. at risk						
<150	56	36	17	9	6	3
150 to 300	64	33	12	3	1	1
>300	27	8	3	1	1	1

Platelet-lymphocyte ratio and multivariate survival

Table 25 outlines the results of a multivariate Cox regression analysis including preoperative PLR as a continuous prognostic variable alongside the histological prognostic factors of relevance. The results indicate that elevated preoperative PLRs continue to exhibit a strong adverse association with survival (p=0.002).

Table 25 - Multivariate Cox analysis of preoperative PLR alongside tumour histology / chemo.

(n=137)	**Hazard ratio (95% CI)**	χ^2	p
PLR	1.003 (1.001 to 1.005)	9.69	**0.002**
Tumour size	1.020 (1.003 to 1.037)	5.21	**0.022**
Poor differentiation	1.522 (1.016 to 2.281)	4.14	**0.042**
Lymph node ratio	6.100 (1.887 to 19.716)	9.13	**0.003**
Adjuvant chemotherapy	0.679 (0.430 to 1.074)	2.74	0.098

Relationship between platelet-lymphocyte ratio and tumour histology

Table 26 outlines a breakdown of median platelet-lymphocyte ratios according to the various histological tumour characteristics. The results demonstrate a trend towards greater platelet-lymphocyte ratios being associated with more invasive histological tumour characteristics (ie. the presence of T3/T4 tumours and vascular / perineural invasion).

Table 26 - Relationship between preoperative PLR and histological tumour characteristics.

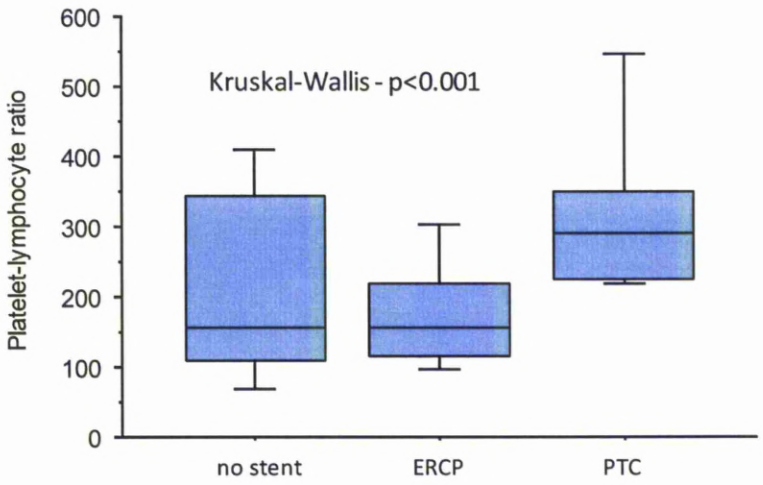
	Median PLR (IQR)	p-value*
Tumour Size:		
≤20mm	151 (111 to 244)	0.597
>20mm	181 (124 to 256)	
T stage:		
T1/T2	139 (95 to 216)	0.063
T3/T4	181 (123 to 261)	
Vascular invasion:		
negative	139 (96 to 187)	**0.004**
positive	182 (131 to 270)	
Perineural invasion:		
negative	106 (97 to 117)	**0.038**
positive	171 (125 to 256)	
Nodal Status:		
negative	187 (131 to 258)	0.658
positive	162 (117 to 251)	
Differentiation:		
well / moderate	158 (116 to 240)	0.150
poor	193 (139 to 289)	

*p-values for Mann-Whitney test

Platelet-lymphocyte ratio and preoperative biliary stenting

There was no overall difference in preoperative platelet-lymphocyte ratios when comparing stented vs unstented patients (Mann-Whitney, p = 0.857). However, patients requiring percutaneous biliary drainage had a significantly greater preoperative platelet-lymphocyte ratio (290 (IQR = 227 to 341)) when compared with patients stented endoscopically (158 (IQR = 117 to 219)) and those who did not undergo biliary stenting (157 (IQR = 112 to 335)) - Kruskal-Wallis, $p < 0.001$ - *fig.44*. There was no significant difference between the latter two groups (Mann-Whitney, p = 0.617).

fig.44 - Box plot to illustrate relationship between preoperative platelet-lymphocyte ratio and biliary stenting.



Discussion

Systemic inflammation is associated with release of a number of inhibitory immunological mediators, most notably interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), which can result in a significant immunosuppressive effect with consequent impaired lymphocyte function (Salazar-Onfray et al, 2007). Pancreatic cancer cells directly secrete these two inhibitory cytokines (Bellone et al, 1999) and lower circulating serum levels of TGF-β2 have been shown to be associated with a more favourable survival outcome in pancreatic ductal adenocarcinoma (Bellone et al, 2006). Lymphocytopaenia has previously been demonstrated to be more strongly associated with pancreatic adenocarcinoma when compared with gastric and colorectal cancer (Romano et al, 2004) suggesting that pancreatic malignancy is associated with a more marked host inflammatory response than other gastrointestinal cancers. In addition to pancreatic cancer commonly exhibiting reduced circulating lymphocyte populations, a reduced number of tumour-infiltrating lymphocytes in resected pancreatic adenocarcinoma specimens have also been found to be associated with poorer survival rates following surgery (Fukunaga et al, 2004). Lymphocyte trapping within peritumoral fibrous tissue is believed to be an additional factor by which pancreatic cancer cells evade immune surveillance (von Bernstorff et al, 2001).

Pancreatic cancer commonly causes a hypercoagulable state resulting in a predisposition to thromboembolic events (Khorana et al, 2004). This is largely attributable to tumor expression of tissue factor which binds to factor VIIa, activating the clotting cascade and promoting thrombin production (Haas et al, 2006). Pancreatic cancer exhibits significant over-expression of tissue factor when compared with normal pancreatic tissue along with upregulation of vascular endothelial growth factor expression, thereby potentiating tumour angiogenesis

(Khorana et al, 2007). Tissue factor expression has also been linked with an adverse prognosis in pancreatic ductal adenocarcinoma (Nitori et al, 2005).

The significance of tumour-platelet interactions within this context is incompletely understood. A number of pro-inflammatory mediators (notably IL-1, IL-3 and IL-6) are known to stimulate megakaryocyte proliferation (Klinger et al, 2002; Alexandrakis et al, 2003), therefore, the association between a relative thrombocytosis and adverse overall survival in pancreatic cancer might be explained on the basis that the platelet count reflects an additional index of systemic inflammation elicited by the tumour. Platelet aggregation and degranulation along with the consequent release of platelet-derived pro-angiogenic mediators within the microvasculature of the tumour could also be an important determinant of tumour growth (Sierko et al, 2004). It has previously been suggested that anti-platelet agents might have an inhibitory effect on the invasive potential of pancreatic cancer cells *in vitro* by down-regulating tumour secretion of matrix metalloproteinase-9 (Suzuki et al, 2004).

The preoperative systemic host immune response as a prognostic factor in resected pancreatic cancer has not previously been extensively evaluated. It has been reported that a more marked pre- and postoperative systemic inflammatory response (as evidenced by an elevated serum C-reactive protein (CRP) level >10mg/l) is associated with a poorer survival following resection for pancreatic ductal adenocarcinoma (Jamieson et al, 2005). Elevated preoperative CRP levels have also been shown to be associated with poorer survival following surgery in other gastrointestinal malignancies (Canna et al, 2005; Crumley et al, 2006). In addition to an elevated CRP, the presence of a neutrophilia and relative lymphocytopaenia are recognised features of the systemic inflammatory response. Few studies to date have investigated the potential prognostic role of preoperative lymphocytopaenia in resected pancreatic cancer

(Yamaguchi et al, 2000; Fogar et al, 2006). A similarly small number of studies have investigated the potential utility of the preoperative platelet count as a prognostic marker in resected pancreatic cancer and the results from these studies have been conflicting (Schwarz et al, 2001; Suzuki et al, 2004a; Brown et al, 2005).

The present study provides further evidence to support the assertion that the preoperative lymphocyte and platelet counts confer significant prognostic information in resected pancreatic ductal adenocarcinoma. The expected inverse correlation between neutrophil and lymphocyte counts was observed, suggesting that a significant proportion of patients with pancreatic ductal adenocarcinoma exhibit some degree of systemic inflammation prior to surgery. Furthermore, the positive correlation between neutrophil and platelet count points towards the preoperative platelet count reflecting an additional index of systemic inflammation. This is perhaps also evidenced by the fact that a preoperative neutrophilia and thrombocytosis were recorded in a similar proportion of the overall patient group.

The results of the preliminary univariate survival analysis indicated that the pre-operative platelet count carried the most significant prognostic information of the three recorded haematological parameters when modelled as a continuous variable within this patient cohort, with the lymphocyte count displaying borderline significance. The platelet-lymphocyte (P/L) ratio was a superior prognostic marker when compared with either individual parameter or the neutrophil-lymphocyte ratio. When categorizing the overall number of patients into three groups according to the preoperative platelet-lymphocyte ratio, Kaplan-Meier analysis also demonstrated a consistent pattern of progressively poorer survival associated with larger platelet-lymphocyte ratios. The median survival associated with a value of >300 appeared to

be comparable with that which would be expected for locally advanced disease (Sultana et al, 2007).

Preoperative intervention for biliary drainage represents a potential confounding factor given the previously described association between CRP and percutaneous biliary procedures. The results also indicated elevated platelet-lymphocyte ratios in the small number of cases requiring PTC. However, there was no significant overall difference in platelet-lymphocyte ratios between stented and unstented patients and given the previous findings indicating no association between stenting and subsequent survival, it is unlikely that that the issue of preoperative biliary drainage is a significant factor in explaining the strong association between preoperative platelet-lymphocyte ratio and overall postoperative survival. The results are consistent with the hypothesis that greater preoperative platelet-lymphocyte ratios reflect a marker of an enhanced host inflammatory response to more locally invasive tumour biology. These results have been published in 2008 (*Appendix B*).

2.5. *The predictive value of the platelet-lymphocyte ratio in determining resectability of suspected pancreatic and peri-ampullary adenocarcinoma*

The finding that the preoperative platelet-lymphocyte ratio exhibited a significant association with invasive histological tumour characteristics prompted an additional analysis in order to ascertain whether this index may represent a marker of tumour resectability alongside CA19-9 in guiding patient selection for staging laparoscopy (Connor et al, 2005). In order to assess this, a cohort of 336 patients with suspected pancreatic or peri-ampullary neoplasms undergoing staging laparoscopy were identified between January 1997 and September 2006. 263 patients from this group went on to laparotomy with a resection rate of 72% (190/263). The median interval between laparoscopy and surgery was 14 (IQR = 7 to 28) days. The histological diagnoses recorded in this group are shown in *Table 27*. 149 patients were male (57%) and the median age was 65 (IQR = 58 to 71) years.

Table 27 - Frequency of tumour type in patients with resectable disease according to CT assessment.

Histology	Frequency
Pancreatic ductal adenocarcinoma	119
Ampullary adenocarcinoma	48
Cholangiocaricnoma	34
Other malignancy	24
Metastatic adenocarcinoma (unconfirmed primary)	25
Presumed peripancreatic malignancy	4
Benign tumours	9
Total	263

A preoperative full blood count with differential white cell count was available in 225 of 263 patients. 188 (84%) of these cases had a full blood count recorded within 2 days of surgery, 22 (10%) within 2-7 days and 15 (6%) within more than 7 days prior to surgery. 216 of 263 patients had preoperative CA19-9 levels recorded. The median interval from the preoperative CA19-9 to date of surgery was 26 (IQR = 15 to 39) days. CA19-9 levels were adjusted for the presence of concurrent obstructive jaundice as previously described. Concurrent bilirubin levels were available in 142 of these cases, 94 (66%) of whom had bilirubin levels of >35 μmol/l. The unadjusted CA19-9 was used in cases where bilirubin data was missing.

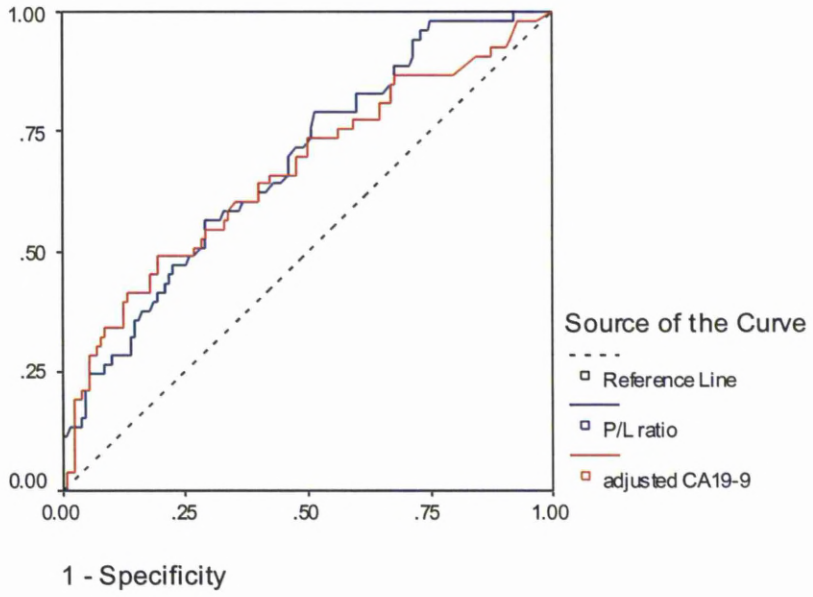
The median adjusted CA19-9 levels and platelet-lymphocyte ratios recorded for patients with resectable disease compared with locally advanced and metastatic disease at laparotomy are shown in *Table 28*. Preoperative CA19-9 levels were greatest in patients with metastatic disease at laparotomy while preoperative platelet-lymphocyte ratios were greatest in those with locally advanced disease.

Table 28 - Comparison of median preoperative adjusted CA19-9 and platelet-lymphocyte (P/L) ratios in resected and inoperable pancreatic and peri-ampullary neoplasms (Kruskal-Wallis).

	Preoperative CA19-9 levels (kU/l)			**Preoperative P/L ratio**		
	n	Median CA19-9 (IQR)	p	n	Median P/L ratio (IQR)	p
Resected tumour	157	144 (27 to 569)		158	147 (113 to 208)	
Locally advanced	36	413 (106 to 4546)	**0.001**	43	202 (146 to 265)	**<0.001**
Metastatic	19	1350 (232 to 2554)		22	176 (139 to 363)	

ROC curves for preoperative adjusted CA19-9 and platelet-lymphocyte ratios in predicting tumour resectability are shown in *fig.45*. The areas under the curve (AUC) recorded for the two prognostic indices were very similar: AUC = 0.67 (95% CI = 0.58 to 0.76) for CA19-9 and AUC = 0.68 (95% CI = 0.60 to 0.77) for platelet-lymphocyte ratio. Using various cut-off values for the platelet-lymphocyte ratio, a level of 150 was found to result in a comparable positive predictive value and specificity when compared with a CA19-9 cut-off value of 150 kU/l.

fig.45 - Receiver operating characteristic (ROC) curves to compare the predictive values of preoperative CA19-9 and platelet-lymphocyte (P/L) ratio in determining peri-ampullary tumour resectability at laparotomy.



The predictive values of CA19-9 levels ≤150 kU/l in determining tumour resectability at laparotomy are shown in *Table 29*. A positive predictive value, negative predictive value, sensitivity and specificity of 83%, 36%, 51% and 73% were recorded respectively (n=216). The predictive values for platelet-lymphocyte ratios ≤150 were broadly comparable with values of 81%, 38%, 51% and 72% respectively (n=225) as shown in *Table 30*.

Table 29 - Contingency table for preoperative CA19-9 levels ≤150 kU/l (or ≤300 kU/l in jaundiced patients) in predicting periampullary tumour resectability at laparotomy (n=216).

		Resectable	Unresectable
Preoperative CA19-9 levels	≤150 kU/l (n=96)	80	16
	>150 kU/l (n=120)	77	43

Predictive values of CA19-9 ≤150 kU/l in determining resectable disease at laparotomy:

Positive predictive value = 83%

Negative predictive value = 36%

Sensitivity = 51%

Specificity = 73%

Table 30 - Contingency table for preoperative platelet-lymphocyte ratios ≤150 kU/l in predicting periampullary tumour resectability at laparotomy (n=225).

		Resectable	Unresectable
Preoperative P/L ratio	≤150 (n=100)	81	19
	>150 (n=125)	77	48

Predictive values of P/L ratio ≤150 in determining resectable disease at laparotomy:

Positive predictive value = 81%

Negative predictive value = 38%

Sensitivity = 51%

Specificity = 72%

The predictive values of using the combined requirement for both CA19-9 and platelet-lymphocyte ratio to be ≤150 are shown in *Table 31* with respective values of 95%, 35%, 28% and 96% (n=183). These combined criteria resulted in a significantly improved specificity over using CA19-9 in isolation (Fisher's exact - $p<0.001$) and a borderline significant improvement in the positive predicitive value for resectability (Fisher's exact - $p=0.065$).

Table 31 - Contingency table for combined requirement of preoperative platelet-lymphocyte (P/L) ratio ≤150 along with CA19-9 levels ≤150 kU/l (or ≤300 kU/l in jaundiced patients) in predicting periampullary tumor resectability at laparotomy (n=183).

		Resectable	Unresectable
Combined CA19-9 and P/L ratio	≤150 for both (n=38)	36	2
	>150 for either (n=145)	94	51

Predictive values of combined score in determining resectable disease at laparotomy:

Positive predictive value = **95%**

Negative predictive value = 35%

Sensitivity = 28%

Specificity = **96%**

If using both CA19-9 and platelet-lymphocyte ratio to guide decision-making regarding the requirement for preoperative laparoscopic staging, 21% (38/183) of laparoscopies would have been avoided with a false positive rate for resectability at laparotomy of only 5% (2/38).

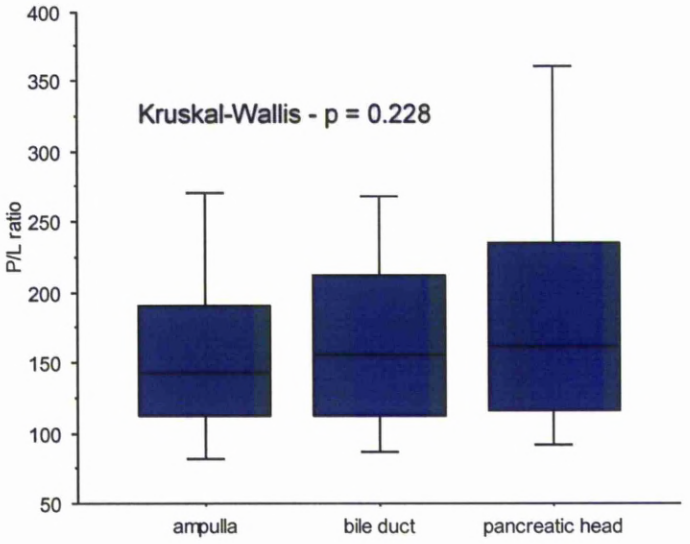
The relationship between the preoperative platelet-lymphocyte ratio and tumour histology in resected pancreatic and peri-ampullary adenocarcinoma

The relationships between the platelet-lymphocyte ratio and tumour resectability and were investigated in a group of 204 resected pancreatic and periampullary adenocarcinomas undergoing pancreatoduodenectomy from this cohort in whom a preoperative full blood count result was recorded. This group comprised 113 patients with pancreatic ductal adenocarcinoma, 53 with ampullary adenocarcinoma and 38 with intra-pancreatic bile duct adenocarcinoma.

Platelet-lymphocyte ratio and origin of primary

There was no significant difference in the median recorded preoperative platelet-lymphocyte ratio when comparing adenocarcinomas arising from the ampulla, intrapancreaic bile duct and head of pancreas (Kruskal-Wallis, p=0.228) - *fig.46*.

fig.46 - Box plot of preoperative platelet-lymphocyte (P/L) ratios in the resected peri-ampullary adenocarcinoma groups (n=204).



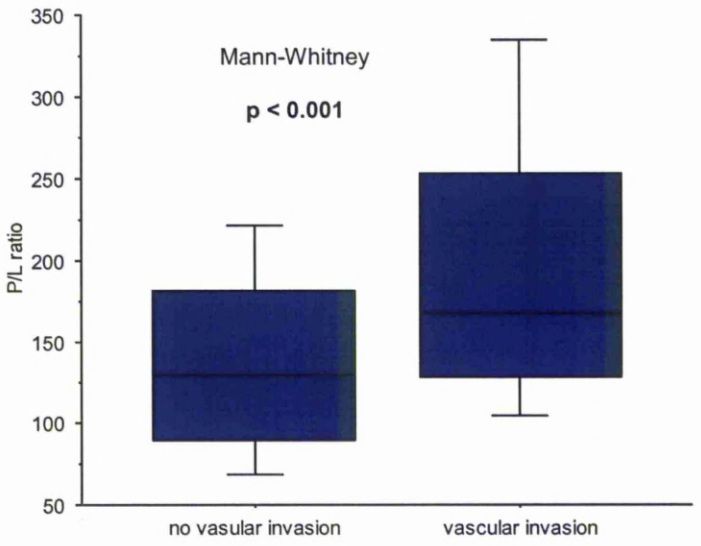
The previously observed association between preoperative platelet-lymphocyte ratio and invasive histological tumour characteristics was further investigated in this cohort of resected pancreatic and periampullary cancers.

Platelet-lymphocyte ratio and vascular invasion

The presence of absence of vascular invasion was recorded in 178 of the 204 resected peri-ampullary adenocarcinoma cases. *fig.47* demonstrates that patients with evidence of vascular invasion on microscopic histological assessment (n=116) had significantly greater

preoperative platelet-lymphocyte ratios than those with no evidence of vascular invasion (n=62) -Mann-Whitney, p<0.001.

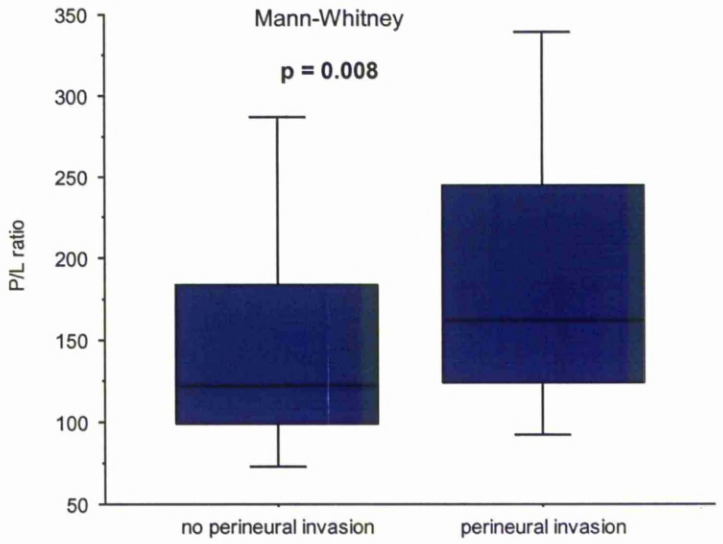
fig.47 - Box plot of preoperative platelet-lymphocyte ratios recorded in peri-ampullary adenocarcinoma cases according to vascular invasion (n=178).



Platelet-lymphocyte ratio and perineural invasion

The presence or absence of perineural invasion was recorded in 181 of the 204 resected peri-ampullary adenocarcinoma cases. *fig.48* similarly demonstrates that cases with perineural invasion (n=149) had a significantly greater median preoperative platelet-lymphocyte ratio when compared with cases with no evidence of perineural invasion (n=32) - Mann-Whitney, p=0.008.

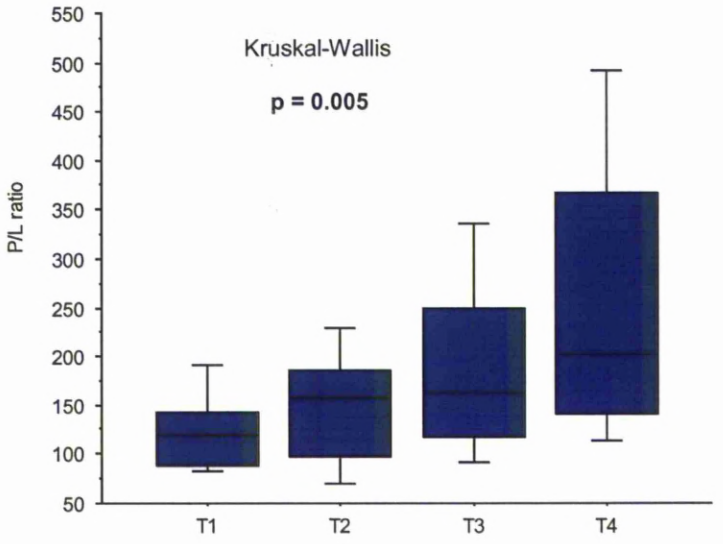
fig.48 - Box plot of preoperative platelet-lymphocyte ratios recorded in peri-ampullary adenocarcinoma cases according to perineural invasion (n=181).



Platelet-lymphocyte ratio and T stage

T stage was recorded in 203 of the 204 resected peri-ampullary adenocarcinoma cases. T1 tumours accounted for 15 cases (7%), T2 tumours accounted for 26 cases (13%), T3 tumours accounted for 154 cases (76%) and T4 tumours accounted for 8 cases (4%). *fig.49* demonstrates that increasing T stage was strongly associated with a greater median preoperative platelet-lymphocyte ratio (Mann-Whitney, p=0.005).

fig.49 - Box plot of preoperative platelet-lymphocyte ratios recorded in peri-ampullary adenocarcinoma cases according to T stage (n=203).



Discussion

Laparoscopic staging has been demonstrated to influence decision-making regarding surgical intervention in approximately 15% of patients with radiologically resectable periampullary malignancy (Minnard et al, 1998; Doran et al, 2004). Use of staging laparoscopy can minimise potentially unnecessary surgical intervention in cases of locally-advanced and metastatic disease missed by CT imaging and facilitate earlier administration of the most appropriate palliative therapy (Nieveen van Dijkum et al, 2003; Ellsmere et al, 2005; Thomson et al, 2006; Doucas et al, 2007). Although endoscopic ultrasound represents a potential alternative staging modality to image tumour relationships with local vasculature along with regional adenopathy (Dewitt et al, 2006), the ability to visually inspect the peritoneal cavity and liver surface to exclude small metastatic deposits represents the principal advantage of laparoscopy over other staging modalities. Staging laparoscopy has

also been demonstrated to be as useful in influencing operative decision-making for periampullary tumors of non-pancreatic origin (Brooks et al, 2002).

Significantly elevated CA19-9 levels have been shown to represent a reliable marker of metastatic disease (Schlieman et al, 2003), but CA19-9 is believed to be relatively less effective at identifying locally advanced disease (Connor et al, 2005). Low preoperative CA19-9 levels have previously been investigated as a potential means of reliably identifying patients with resectable periampullary tumours at laparotomy, thereby avoiding the requirement for supplementary staging in all patients (Schlieman et al, 2003; Connor et al, 2005; Karachristos et al, 2005;). This presents the opportunity to make more judicious use of staging laparoscopy, which is particularly relevant in centres where laparoscopic staging is routinely conducted on separate theatre sessions prior to laparotomy.

The results of the present study indicate that the preoperative platelet-lymphocyte ratio correlates with features of local tumor invasiveness in cases of resected peri-ampullary adenocarcinoma. Increasing T-stage along with the presence of vascular and perineural invasion were associated with a trend towards greater median preoperative platelet-lymphocyte ratios in the overall patient cohort. This finding further indicates that the platelet-lymphocyte ratio is more significantly influenced by local tumour infiltration rather than overall tumour burden. This observation is also supported by the fact that a greater median platelet-lymphocyte ratio was observed in patients with locally advanced periampullary tumors when compared with those with metastatic disease. This contrasts with the results seen for CA19-9 which demonstrated greater median CA19-9 values in patients with metastatic disease at laparotomy, a finding consistent with previous studies (Schlieman et al, 2003).

Analysis of the ROC curves indicates that the overall predictive value of the preoperative platelet-lymphocyte ratio is comparable with that seen for CA19-9 levels. The contingency tables for CA19-9 and platelet-lymphocyte ratio also resulted in a broadly comparable positive predictive value and specificity for both. The combined use of CA19-9 levels $\leq$150 kU/l (or $\leq$300 kU/l in jaundiced patients) and a platelet-lymphocyte ratio $\leq$150 resulted in a significant improvement in the ability to identify a low-risk group for unresectable disease at laparotomy with a positive predictive value for resectability of 95% and a specificity of 96%. Use of these combined criteria for selective use of staging laparoscopy would have resulted in 38 of 183 laparoscopies (21%) being avoided with a false positive rate of only 5% - ie. only 5% of patients going straight to laparotomy with unresectable disease. The poor negative predictive value and sensitivity for CA19-9 and platelet-lymphocyte ratios indicate that neither parameter can reliably predict *unresectable* periampullary tumors and, as such, investigating the predictive values of these parameters in the patient group with convincing evidence of advanced disease diagnosed at CT or laparoscopy would not result in any information which would alter decision-making regarding surgery.

It has previously been estimated that less than 5% of the overall population lack the Lewis antigen glycosyl transferase enzyme required to synthesize CA19-9 (Itzjowitz et al, 1986). This represents a potential confounding factor in interpreting the predictive values associated with CA19-9. However, only 5 out of 216 cases (2.3%) in the present study for whom a preoperative CA19-9 was recorded had unrecordable CA19-9 levels (<2 kU/l). These cases were included in the analysis in order to avoid potential bias, but this issue is unlikely to have a significant impact on the validity of the CA19-9 predictive values recorded in this study.

In summary, this is the first study to report an association between preoperative inflammation and periampullary cancer resectability. The preoperative platelet-lymphocyte ratio was found to be associated with both macroscopic and microscopic features of periampullary tumor invasiveness and appears to be a more effective marker of locally advanced disease than CA19-9. Use of both CA19-9 and platelet-lymphocyte ratio in risk stratifying patients with suspected peri-ampullary malignancy for staging laparoscopy resulted in a significant improvement in the ability to identify those patients in whom supplementary staging can be safely avoided. The results of this study suggest that the preoperative platelet-lymphocyte ratio merits prospective evaluation alongside CA19-9 in this setting. These findings have been published in 2008 (*Appendix B*).

2.6. *Combined prognostic score*

The four preoperative parameters of univariate significance (ie. albumin, CRP, CA19-9 and platelet-lymphocyte ratio) were included in a multivariate Cox regression analysis (*Table 32*). Due to incomplete data for each variable, this analysis included a total of 98 patients.

Table 32 - Multivariate Cox analysis of preoperative prognostic factors.

(n=98)	Hazard ratio (95% CI)	χ^2	p
albumin	0.955 (0.908 to 1.005)	3.17	0.075
CRP	0.996 (0.983 to 1.009)	0.33	0.567
lnCA19-9	1.182 (1.031 to 1.355)	5.74	**0.017**
Platelet-lymphocyte ratio	1.003 (1.001 to 1.005)	6.83	**0.009**

The preoperative CA19-9, platelet-lymphocyte ratio and albumin were used to generate a combined preoperative prognostic score according to the following formula (Piantadosi, 2005):

$$r_i = \sum_{j=1}^{p} \beta_j X_{ij}$$

r_i = risk score

j = number of covariates

i = number of patients

X = individual value for covariate j

β_j = Cox regression coefficient for covariate j

Data for the CA19-9, platelet-lymphocyte ratio and albumin were complete for 122 cases. Within this patient group, the combined score exhibited a stronger relationship with survival on a continuous basis than any of the individual parameters (*Table 33*).

Table 33 - Univariate comparative Cox analysis of combined preoperative prognostic score.

(n=122)	**Hazard ratio (95% CI)**	χ^2	p
albumin	0.945 (0.908 to 0.983)	7.90	**0.005**
platelet-lymphocyte ratio	1.002 (1.001 to 1.004)	8.40	**0.004**
lnCA19-9	1.228 (1.087 to 1.386)	10.92	**0.001**
Combined preoperative score	2.020 (1.476 to 2.764)	19.29	**<0.001**

The same principle was used for the three histological variables of significance (ie. tumour size, differentiation and lymph node ratio) to generate a combined histological score. The histological data were complete for 157 cases within this group (*Table 34*).

Table 34 - Univariate comparative Cox analysis of combined histological prognostic score.

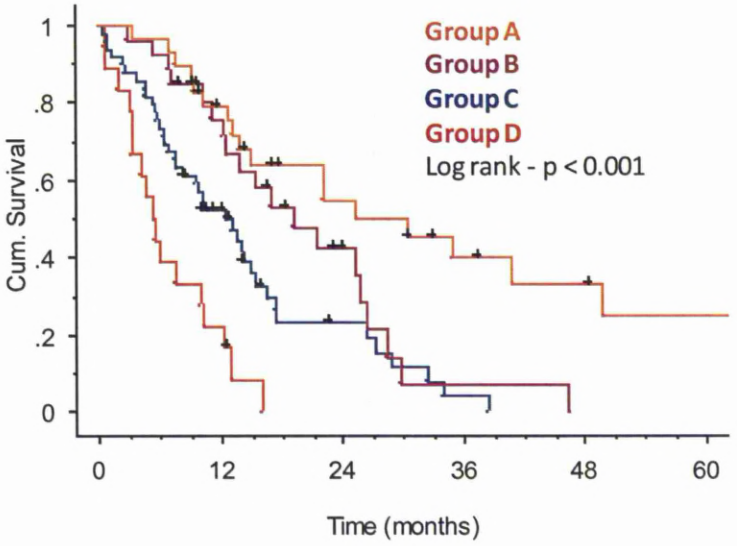
(n=157)	**Hazard ratio (95% CI)**	χ^2	p
tumour size	1.021 (1.006 to 1.037)	7.08	**0.008**
poor differentiation	1.663 (1.149 to 2.407)	7.26	**0.007**
lymph node ratio	4.631 (1.916 to 11.195)	11.59	**<0.001**
Combined histological score	2.305 (1.620 to 3.281)	21.54	**<0.001**

An overall prognostic index was calculated on an additive basis using both scores (*Table 35*). This overall score allowed a superior degree of risk stratification into four groups (*fig.50*).

Table 35 - Univariate survival analysis of combined overall prognostic score as a continuous and categorical variable (n=122).

(n=122)	Median survival (95% CI) months	Hazard ratio (95% CI)	X^2	p-value
Overall score (4 groups):		-	36.84	**<0.001**
Group A	30.4 (15.0 to 49.8)	-	-	-
Group B	19.2 (12.6 to 25.8)	2.124 (1.069 to 4.219)	4.63	**0.032**
Group C	12.6 (8.2 to 15.5)	3.463 (1.872 to 6.404)	15.68	**<0.001**
Group D	5.8 (4.7 to 10.4)	9.598 (4.507 to 20.443)	34.38	**<0.001**
Combined overall score:				
continuous	-	2.050 (1.590 to 2.643)	30.66	**<0.001**

fig.50 - Kaplan-Meier survival curves stratified according to combined overall prognostic score.



No. at risk						
Group A	29	21	12	7	5	3
Group B	26	17	7	1	0	0
Group C	49	21	6	1	0	0
Group D	18	4	0	0	0	0

Using the formula outlined on page 148, the combined prognostic score was calculated for each patient. This score ranged from -1.42 to 2.39 with a median of 0.64. The overall prognostic score was used to divide patients into four groups according to the maximum degree of risk stratification possible (*fig.50*). The cut-off values used to categorise patients from this combined score to generate the above survival curves were as follows:

- Group A = < -0.05
- Group B = -0.05 to 0.60
- Group C = 0.61 to 1.60
- Group D = >1.60

2.7. *Preoperative factors and patient selection for adjuvant therapy*

An additional analysis was undertaken in order to identify any potential preoperative factors which were predictive of patient selection for adjuvant therapy following resection. This included patients randomised to ESPAC-1 or ESPAC-3 (to either treatment or observation arms) and the small number of patients who received off-trial adjuvant treatment. *Table 36* outlines the results of univariate logistic regression with patient selection for adjuvant therapy as the dependent variable. All factors with the exception of gender were included as continuous variables. Hence, an odds ratio of 0.930 for patient age (regression coefficient of -0.073) indicates a reduced likelihood of selection for adjuvant treatment by a factor of 0.930 for each increase in age by one year. Therefore, a 60-year old patient would be 4 times more likely to be selected for adjuvant treatment than an 80-year old patient (ie. $1 \div e^{((80-60) \times -0.073)} = 4.307$).

Table 36 - Logistic regression analysis of factors predictive of patient selection for adjuvant therapy.

	Odds ratio (95% CI)	χ^2	**p**
Univariate			
age	0.930 (0.892 to 0.970)	11.60	**<0.001**
gender (M)	1.100 (0.572 to 2.114)	0.08	0.776
albumin	1.115 (1.045 to 1.190)	10.79	**0.001**
bilirubin	0.999 (0.995 to 1.003)	0.27	0.603
platelet-lymphocyte ratio	0.994 (0.990 to 0.998)	8.03	**0.005**
lnCA19-9	0.905 (0.755 to 1.086)	1.14	0.285
Multivariate			
age	0.925 (0.877 to 0.975)	8.25	**0.004**
platelet-lymphocyte ratio	0.995 (0.990 to 1.000)	4.61	**0.032**
albumin	1.085 (1.001 to 1.171)	3.98	**0.046**

This analysis indicates that patient age, preoperative platelet-lymphocyte ratio and albumin levels were significant independent predictors of patient selection for adjuvant therapy following resection. Younger patients were significantly more likely to be selected (ie. odds ratio < 1), while patients with lower preoperative platelet-lymphocyte ratios and higher albumin levels were also significantly more likely to be selected for adjuvant treatment. These factors were independent on multivariate logistic regression.

Patient's age, their overall recovery from surgery and functional performance status within the first 4 to 6 postoperative weeks are the main factors which determine patient selection as potential candidates for adjuvant treatment. The results from the above analysis indicate that the preoperative host inflammatory response, as measured by the platelet-lymphocyte ratio, may be a significant contributory factor to patient's initial postoperative recovery as well their overall subsequent survival as evidenced from the observation that patients with increasing preoperative platelet-lymphocyte ratios were proportionally less likely to go on to receive adjuvant therapy. The association between albumin levels and selection for adjuvant treatment suggests that the preoperative nutritional status of patients may also be a significant factor in influencing early postoperative recovery (Giger et al, 2007). Furthermore, the previous multivariate Cox analyses demonstrated that the associations between these two preoperative parameters and postoperative survival were mutually independent of adjuvant treatment received. These observations merit prospective evaluation in a larger cohort of patients.

3. SYSTEMATIC REVIEW & META-ANALYSIS OF MOLECULAR MARKERS

3.1. METHODS

Search Strategy

From a preliminary analysis of existing review articles of molecular prognostic studies in pancreatic cancer, the most widely investigated molecular markers were selected for analysis. Literature was searched using the MEDLINE, EMBASE and Web of Science search engines. Search criteria were based on the relevant marker selected and included appropriate synonyms:

- eg. (p16 OR p16INK4a OR p16*) AND (pancreas OR pancreatic) AND (survival OR prognostic OR prognosis).

In addition, the ISI Proceedings search engine was also searched in order to identify unpublished studies presented at international conferences / meetings. This was supplemented by conducting an abstract search of the American Society of Clinical Oncology (ASCO) website, the American Association of Cancer Research (AACR) website and the British Society of Gastroenterology (BSG) websites for relevant unpublished studies.

Selection criteria

A standardised eligibility form was generated to determine which studies were eligible for selection for potential meta-analysis. All searches were conducted in December 2009. This process was conducted by two independent reviewers (Richard Smith and Paula Ghaneh). Where part or all of the same patient series was included in more than one publication, only the more recent or most complete study was included in the analysis in order to avoid duplication of the same survival data. In studies where reported survival data were incomplete but the other relevant inclusion criteria were fulfilled, supplementary survival

data were requested from the authors of the relevant study. The criteria for selection are listed below:

- only resected pancreatic adenocarcinoma cases included in prognostic analysis.
- only studies conducting immunohistochemical analysis of resected primary tumour material.
- only studies where a univariate survival analysis was conducted for the relevant prognostic marker (overall survival).
- only studies where the marker of interest was dichotomised (ie. positive vs. negative immunostaining). Studies with three or more risk groups were excluded.

Quality assessement

An assessment of methodological quality was also performed for each piece of literature selected. A standardised form was used which assessed the important components of a well-conducted prognostic study. These criteria were adapted from existing literature (Hayden et al. 2006; Steels et al. 2001) and were used to define 20 individual study characteristics which were deemed to be key factors to report in an immunohistochemical prognostic study (*Table 1*). For any criterion not fulfilled according to the information outlined in the article, one point was deducted from a maximum of 20 and the final score was recorded as a percentage. The eligibility criteria and quality scoring were assessed by two independent investigators (RS and PG). Any disagreement was resolved by discussion.

Table 1 - Methodological scoring criteria used.

Study group	
• Study population adequately described:	
○ Gender / Age	1 point
○ Histology	1 point
• Period of recruitment:	1 point
• Inclusion / exclusion criteria used:	1 point
Study attrition	
• >90% of cases identified included in final analysis:	1 point
• Reasons for attrition / loss to follow-up given:	1 point
• Peri-operative mortality details:	1 point
Scientific methodology	
• IHC methodology outlined:	
○ Details of $1^{\circ}/2^{\circ}$ Abs used:	1 point
○ Concentration of 1° Abs used:	1 point
○ Positive / negative controls outlined:	1 point
• Description of scoring technique:	
○ >1 independent scorer:	1 point
○ Scorers blinded to clinical data:	1 point
○ Criteria for positivity clearly outlined:	
▪ Distribution (cytoplasm vs. membranous vs. nuclear):	1 point
▪ % positive cells for immunostaining classification:	1 point
Confounding factors considered	
• Adjuvant therapy details provided:	1 point
• Histological breakdown according to IHC staining:	1 point
Statistical analysis	
• HR (confidence interval) provided:	1 point
• Exact p-value quoted:	1 point
• Numbers at risk for Kaplan-Meier curves:	1 point
• Number of censored cases recorded:	1 point

Data extraction

Data were extracted by RS. A study was considered significant if the p-value for the statistical comparative test used (eg. log rank) was less than 0.05. Studies were also categorised according to the direction of the survival relationship observed (ie. whether positive staining of the marker of interest conferred an adverse or more favourable survival outcome). The primary outcome measure was overall survival (ie. date of resection to date of death). Additional details were also collected in order to identify potential sources of heterogeneity. These included the specific primary antibody (and dilution) used for immunohistochemistry, the scoring criteria used to define positive staining and relevant clinico-pathological data. The relationships between positive staining and resected histopathological tumour characteristics were also recorded as a secondary end-point. A standardised data extraction form was used in order to obtain the relevant survival data required for either direct or indirect calculation of the log hazard ratio and variance (*Table 2*).

As few studies directly quote the log hazard ratio and variance (or standard error) for survival analyses, an indirect approach is usually required in order to estimate these values for quantitative aggregation of survival data (Parmar et al. 1998, Tierney et al. 2007, Williamson et al. 2002). Where the HR and 95% confidence interval were quoted, these values were used to generate the log hazard ratio and variance. If not reported, the log rank p-value (or associated chi-squared statistic) and the number of reported events were instead used to calculate these values. If survival data were only presented graphically, Kaplan-Meier survival curves were used to estimate these values (Parmar et al. 1998, Tierney et al. 2007). In a small number of studies, raw survival data were presented in a tabulated format allowing direct estimation of the log hazard ratio and variance using standard statistical software (Statview). If insufficient survival data were presented to allow any indirect estimation of log hazard ratios in an otherwise eligible study, the authors of the relevant paper were contacted

in order to request supplementary data. The HR calculation Excel spreadsheet developed by Tierney et al was used to generate the HR and variance from the available survival data (www.biomedcentral.com/content/supplementary/1745-6215-8-16-S1.xls). The logHR and variance for individual studies were entered into RevMan 4.2 (Cochrane collaboration, Oxford, UK) and pooled using an inverse variance approach. Hazard ratios were calculated for *positive* expression of each molecular marker (ie. a HR > 1 reflecting *adverse* survival associated with positive immunostaining while a HR < 1 reflects *favourable* survival associated with positive staining).

Heterogeneity and Bias

Heterogeneity was assessed using a χ^2 test for heterogeneity with a p-value of < 0.10 taken to reflect the presence of significant heterogeneity. The I^2 statistic was calculated to quantify the degree of heterogeneity (Higgins et al, 2002). A p-value of <0.050 was taken to reflect significance for all other analyses. Due to the relatively limited number of studies included in each analysis, a random-effects approach was used in all cases. Potential sources of heterogeneity between studies were explored (ie. variation in IHC methodology, differences in resected histological tumour characteristics, etc). Publication bias was assessed by analysis of the inverted funnel plots generated in RevMan 4.2. An inverted funnel plot represents a plot of the HR against the standard error of the log HR for each included study. Significant asymmetry about the pooled HR estimate is indicative of publication bias. A visual inspection of each plot was supplemented by calculation of the p-value for Egger's regression (StatsDirect). Funnel plots were not generated when the total number of included studies was small (less than five). Continuous data were compared using Spearman's rank correlation with two-sided Mann-Whitney testing for categorical data.

3.2. RESULTS & DISCUSSION

3.2.1 VEGF Studies

Selection Criteria

The following search criteria were used:

- ('VEGF' OR 'vascular endothelial growth factor' OR 'VEGF*') AND ('pancreas' OR 'pancreatic') AND ('survival' OR 'prognosis' OR 'prognostic').

Abstracts were initially checked for relevance and the full article was retrieved for all potentially eligible studies. Where the same patient series was included in more than one publication, only the more recent or most complete study was included in the analysis in order to avoid overlap. Only studies investigating the prognostic value of VEGF-A expression were included (this represents the most widely investigated and biologically relevant VEGF isoform).

Results

The initial search returned a total of 255 studies. Following review of these abstracts, 20 potentially relevant studies were identified with publication dates ranging from 1997 to 2008. No relevant unpublished abstracts were identified. Following review of the full papers, nine were excluded for the following reasons: duplicated series of patients (Ikeda et al, 1999; Niedergethmann et al, 2000; Tang et al, 2001), only VEGF-C and/or VEGF-D analysed (Kurahara et al, 2004; Zhang et al, 2007), no dichotomised univariate survival analysis reported (Ellis et al, 1998; Fujioka et al, 2001), mix of resected and unresected cases included in survival analysis (Chung et al, 2006), only VEGF receptor status analysed (Büchler et al, 2002). None of the eligible studies described a prospective design and archived paraffin-embedded primary tumour material was utilised for immunohistochemistry in all cases.

The 11 eligible studies included a total of 767 patients with a median number of 62 patients per study (range = 19 to 142). *Table 3* outlines the demographic, clinico-pathological, methodological and outcome characteristics of these studies. Five studies reported a significant adverse association between VEGF expression and survival on univariate analysis. The median quality score was recorded as 70% (range = 60% to 95%). There was no significant difference in median quality scores between significant and non-significant studies (Mann-Whitney, p=0.516). Similarly, there was no significant correlation between study size and quality scores (Spearman, rho = 0.139, p=0.698).

Meta-analysis

fig.1 illustrates the Forrest plot for the survival data. Significant heterogeneity was demonstrated according to Cochran's chi-squared test (χ^2 = 22.08, p=0.01). The combined HR was recorded as 1.51 (95% CI = 1.18 - 1.92) indicating that positive immunostaining for VEGF was significantly associated with adverse survival in the pooled patient group. When assessing the funnel plot for this analysis (*fig.2*), the data points approximated a symmetrical distribution indicating that publication bias is unlikely to be a significant confounding factor in describing this relationship. Egger's regression for this analysis demonstrated a non-significant p-value (p = 0.269).

Table 3 - Methodological and clinico-pathological data for eligible VEGF studies.

	Year	n	HR (95% CI)	Significant	1° Ab (+ dilution)	VEGF +ve	IHC % cut-off	Male	Age	N1	T3/T4	Well	Mod.	Poor	Adjuvant therapy
Itakura et al	1997	75	1.12 (0.69-1.82)	No	NC (30μg/ml)	48 (64)	>10%	46 (61)	62	47 (63)	43 (57)	13 (17)	44 (59)	18 (24)	NS
Fujimoto et al	1998	50	0.78 (0.44-1.40)	No	Santa Cruz (1:200)	28 (40)	NS	28 (56)	62	29 (58)	34 (68)	9 (18)	31 (62)	10 (20)	NS
Seo et al	2000	142	1.46 (1.02-2.09)	Yes	Santa Cruz (NS)	94 (66)	>30%	79 (56)	64	95 (67)	NS	NS	NS	NS	NS
Ikeda et al	2001	48	2.74 (1.44-5.20)	Yes	Santa Cruz (1:200)	31 (65)	>10%	37 (77)	64	24 (50)	40 (83)	15 (31)	28 (58)	5 (11)	48 (100)
Knoll et al	2001	19	2.37 (0.88-6.40)	No	R&D Systems (1:200)	13 (68)	>5%	11 (58)	58	18 (95)	1 (5)	1 (5)	12 (63)	6 (32)	0 (0)
Niedergethman et al	2002	70	2.48 (1.22-5.05)	Yes	Santa Cruz (1:200)	28 (40)	>10%	42 (60)	63	41 (59)	NS	25 (36)		45 (64)	22 (31)
Kuwahara et al	2003	55	2.08 (1.12-3.88)	Yes	Santa Cruz (1:200)	39 (71)	>50%	34 (62)	64	30 (55)	40 (73)	13 (24)	33 (60)	9 (16)	NS
Lim et al	2004	72	0.82 (0.49-1.37)	No	Santa Cruz (1:2000)	23 (32)	>10%	43 (60)	60	38 (53)	59 (82)	14 (19)	44 (61)	14 (19)	26 (36)
Khorana et al	2005	124	1.30 (0.87-1.95)	No	Zymed (1:50)	70 (56)	>5%	69 (56)	67	56 (45)	69 (58)	23 (19)	52 (43)	45 (38)	88 (79)
Tang et al	2006	50	1.46 (0.84-2.54)	No	NS (2μg/ml)	25 (50)	>10%	25 (50)	63	39 (78)	25 (50)	15 (30)	31 (62)	4 (8)	NS
Ai et al	2008	62	2.34 (1.41-3.89)	Yes	Neomarkers (NS)	37 (60)	>10%	36 (58)	65	49 (79)	32 (52)	17 (27)	15 (24)	30 (48)	0 (0)

% in parentheses unless otherwise stated. NS = not specified. NC = non-commercial.

fig.1 - Forrest plot to assess overall effect of VEGF expression on survival.

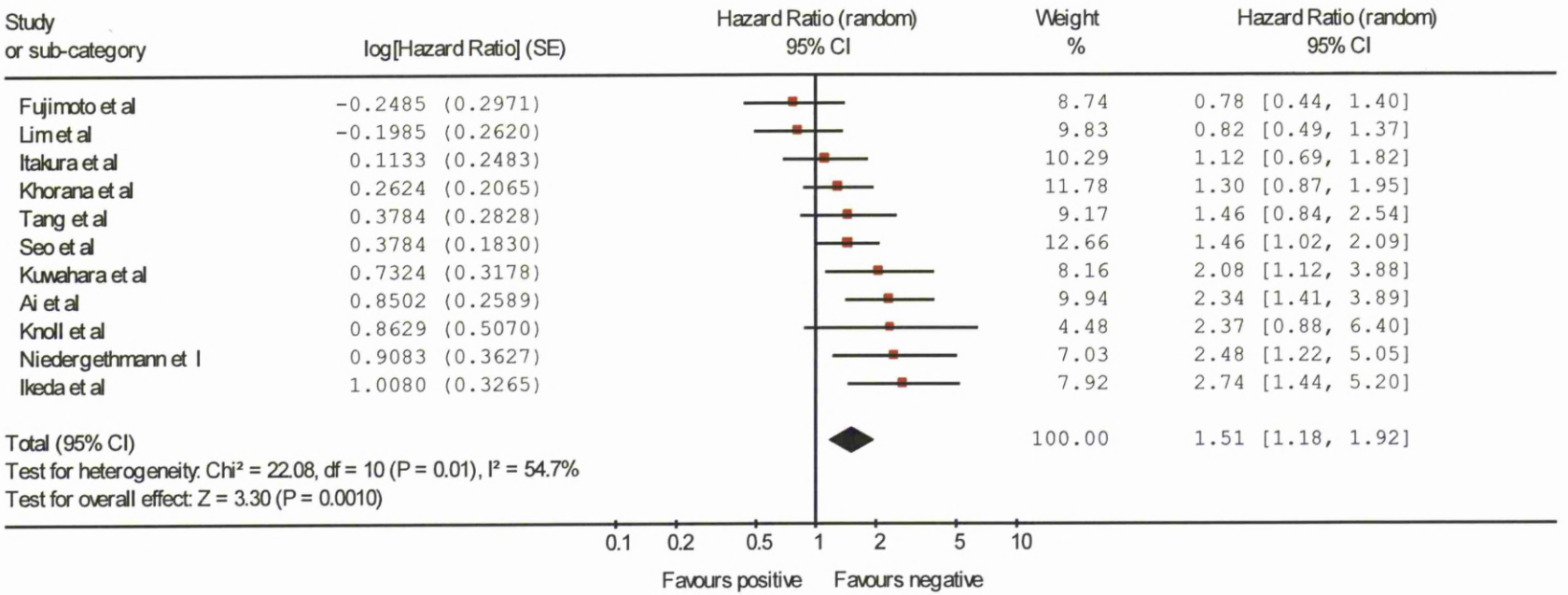
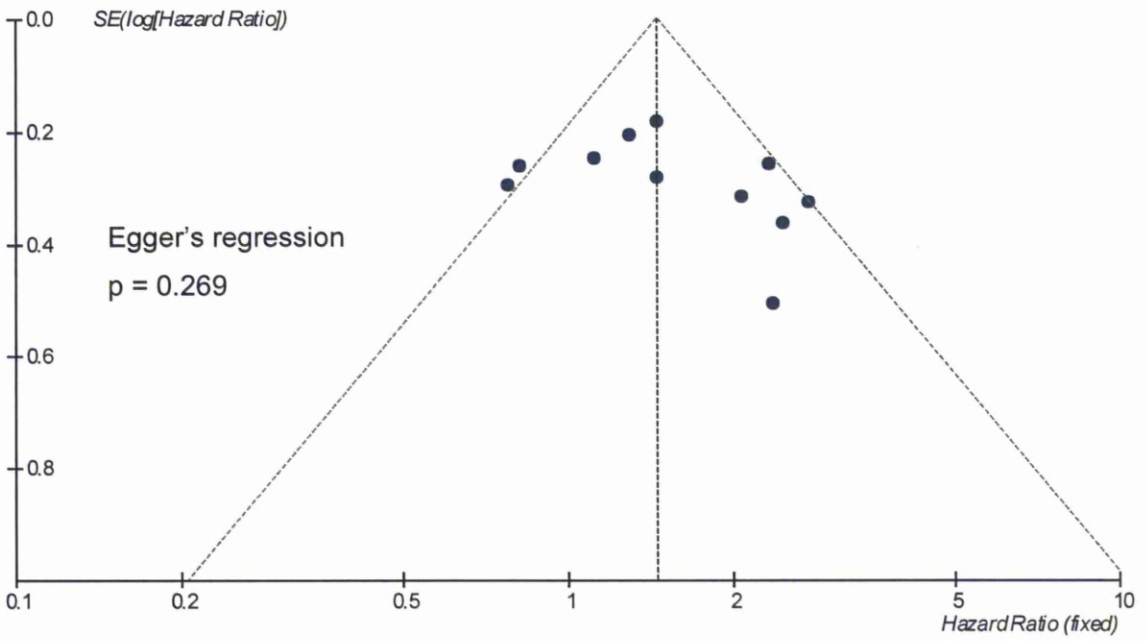


fig.2 - Inverted funnel plot to assess potential publication bias.



Potential sources of heterogeneity

The median proportion of patients classified as VEGF positive in the included studies was recorded as 60% (range = 32% to 71%). The proportion of VEGF positive cases reported in each study failed to exhibit any correlation with the assessment of methodological quality (Spearman, p=0.491) or the % cut-off used to define positive immunostaining (Spearman, p=0.388). No automated immunohistochemical scoring was described in any of the included studies.

Only two studies (Lim *et al*, 2004; Fujimoto *et al*, 1998) reported an opposite survival trend associated with VEGF expression (ie. more favourable survival in VEGF positive tumours). However, neither of these studies approached statistical significance (p=0.448 and p=0.400 respectively). Only six studies reported the proportion of patients who received any form of adjuvant therapy (*Table 2*) and administered treatment modalities included a mix of both chemotherapy and chemoradiation. Importantly, no studies reported any policy of selection of

patients for adjuvant therapy based on VEGF tumour expression as immunohistochemical evaluation was undertaken retrospectively in all cases. No studies reported use of any neoadjuvant therapy and only a single study reported use of intra-operative radiotherapy (Ikeda et al, 2001).

Most studies included a breakdown of VEGF staining according to histological tumour characteristics. *Table 4* outlines the cumulative data to describe the relationship between VEGF expression and nodal status, T stage and tumour grade in the meta-analysed studies where available. These results suggest a significant relationship between positive VEGF immunostaining and an increased likelihood of T3/T4 tumours ($\chi^2 = 5.63$, p=0.018). Despite a similar trend towards a greater proportion of N1 tumours associated with positive VEGF expression, this finding did not reach significance ($\chi^2 = 2.67$, p=0.102).

Table 4 - Pooled data to demonstrate association between VEGF expression and resected histopathological tumour characteristics.

	VEGF positive	**VEGF negative**	**p**
Nodal status:			
N0	132	115	0.102
N1	237	158	
T stage:			
T1/T2	77	72	**0.018**
T3/T4	136	76	
Tumour grade:			
Well	75	54	0.622
Moderate	136	121	
Poor	60	50	

Pooled data was available for nodal status in 9 studies (n=642), for tumour grade in 8 studies (n=496) and T stage in 6 studies (n=361).

Of the five studies which reported positive VEGF expression as a significant adverse prognostic variable, only three conducted some form of multivariate analysis. These three analyses included a variety of disparate covariates alongside VEGF. However, each reported that VEGF status retained statistical significance. Due to the lack of studies reporting multivariate analyses, no attempt was made to use any adjusted survival data as part of this meta-analysis.

Discussion

Previous meta-analyses of studies investigating the immunohistochemical expression of VEGF as a marker of prognosis have been published for various malignancies including head and neck (Kyzas et al, 2005), colorectal (Des Guetz et al, 2006) and lung cancer (Delmotte et al, 2002), all reporting a significant correlation between VEGF expression and poor survival when pooling data from individual studies. To date, no such meta-analysis has been undertaken for prognostic studies evaluating VEGF in pancreatic cancer.

The results from the present study demonstrate that, despite variability between eligible studies as to the relative prognostic impact of VEGF expression in resected pancreatic adenocarcinoma, the overall observed survival trend is concordant with that reported for other malignancies as outlined above. When comparing the value for the pooled HR identified in the present study (1.51 (95% CI = 1.18 to 1.92)) with the studies referenced above, the order of magnitude for this effect is also broadly comparable for that quoted for both lung cancer (1.48 (95% CI = 1.27 to 1.72)) and colorectal cancer (1.65 (95% CI = 1.27 to 2.14)). The analysis of the pooled data indicated that there was no difference between significant and non-significant studies when comparing the allocated scores for

methodological quality. This finding indicates that a meaningful aggregation of survival data can be conducted for both significant and non-significant studies.

When considering the nine studies which were excluded from the analysis, three were removed due to a duplicated cohort of patients included elsewhere and a further three were removed due to VEGF-C, VEGF-D or VEGF receptor status being the immunohistochemical markers of interest. Therefore, the outcomes of these six studies cannot be meaningfully compared with the 11 pooled studies. When evaluating the remaining three studies excluded due to either the lack of a dichotomised univariate survival analysis (Ellis et al, 1998; Fujioka et al, 2001), or mix of resected and unresected cases (Chung et al, 2006), all three studies reported a non-significant relationship between VEGF expression and survival. However, two of these three studies reported the direction of the survival effect to favour lower levels of VEGF immunostaining, in keeping with the general survival trend observed for the aggregated data.

Significant heterogeneity was observed when analysing the logHR estimates from the eligible studies. When evaluating the relevant methodological and clinico-pathological characteristics of each study, a number of potential sources of heterogeneity in study methodology were observed. Nine studies reported use of commercially available anti-VEGF primary antibodies, all of which exhibit broadly comparable binding characteristics with the common splice variants of VEGF-A. One study (Itakura et al, 1997) reported use of a non-commercial VEGF-A specific primary antibody and one study (Tang et al, 2006) did not elaborate on the origin of the anti-VEGF-A antibody used. When analysing the concentrations of primary antibody utilised, most studies reported comparable dilution ratios. However, the concentration was not specified in two studies. This issue is potentially relevant for the study

reporting use of the lowest primary antibody dilution (Lim et al, 2004) as this was one of only two studies which indicated a contradictory prognostic effect (non-significant) when compared with the overall group (ie. a trend towards adverse survival with negative VEGF immunostaining).

When reviewing the immunohistochemical criteria used for VEGF scoring, the majority of studies reported a scoring system based on cytoplasmic staining of tumour cells. Where the distribution of immunostaining used for scoring was not explicitly stated in the text (ie. cytoplasmic, membranous, nuclear, stromal, etc), the figures of representative VEGF staining presented in the relevant studies were strongly indicative of cytoplasmic staining being used to define positive VEGF expression in cancer cells. All studies with one exception utilised a system of dichotomising patients according to the percentage of positively-stained cells present. The % cut-off values ranged from >1% to >50% of cancer cells. However, six out of ten studies utilised a standard cut-off value of >10%. Despite the range of values used to define VEGF positivity across the included studies, there was no evidence of any significant association between the % cut-off value used and the corresponding proportion of VEGF positive patients reported. Furthermore, if only including the six studies using a standardised cut-off value of >10% for meta-analysis, the significance of the association between VEGF staining and adverse survival was unchanged (HR = 1.62 (95% CI = 1.09 to 2.40) - random effects). These observations suggest that differences in the specific scoring criteria used for immunohistochemical classification are unlikely to have a significant confounding effect in describing the underlying relationship between VEGF expression and survival observed for the overall group.

The combined analysis to investigate the relationship between reported VEGF positivity and histological tumour characteristics (*Table 3*) indicated a significant association between VEGF expression and likelihood of more invasive tumours (ie. T3/T4). This finding was also reported by two individual studies (Itakura et al, 1997; Tang et al, 2006). However, only six of the eleven eligible studies (representing less than half the overall patient group) presented sufficient data to determine the exact proportion of T3/T4 tumours stratified by VEGF expression and consequently, this result should be interpreted with caution. There was no demonstrable association between VEGF expression and either tumour grade or nodal status when analysing the available pooled data. Of the three studies identified which investigated VEGF-C and/or VEGF-D alongside VEGF-A expression (Tang et al, 2006) or in isolation (Kurahara et al, 2004; Zhang et al, 2007), all three demonstrated a significantly greater proportion of N1 cases associated with positive VEGF-C/D expression. In contrast, none of these studies found a significant association between tumour stage and VEGF-C/D positivity. These findings are generally consistent with the accepted roles for VEGF-A in mediating growth and local tumour infiltration and VEGF-C/D in promoting lymphatic invasion (Achen et al, 2008).

The eleven studies which fulfilled the eligibility criteria exhibited a comparable mix of significant and non-significant studies. When analysing the funnel plot for the pooled survival data, the distribution of data points was approximately symmetrical. These findings indicate that publication bias is unlikely to represent a significant confounding factor in the interpretation of this meta-analysis.

3.2.2. bcl-2 studies

Selection Criteria

The following search criteria were used:

- ('bcl-2' OR 'bcl2' OR 'bcl' OR 'bcl*) AND ('pancreas' OR 'pancreatic') AND ('survival' OR 'prognosis' OR 'prognostic')

Results

The initial search returned a total of 232 abstracts of which 16 potentially eligible articles were retrieved. A total of 11 were rejected for the following reasons; duplicated series of patients (Nio et al, 2001), mix of resected and unresected cases included (Ohshio et al, 1998; Mäkinen et al, 1998; Gansauge et al, 1998; Hu et al, 1999), inclusion of ampullary tumours (Sinicrope et al, 1996), no dichotomised univariate survival analysis conducted (Evans et al, 2001; Stipa et al, 2002; Sun et al; 2002), insufficient survival data reported for indirect estimation of logHR and variance (Friess et al, 1998; Campani et al, 2001). The latter two authors were contacted to request supplementary survival data. However, this information was either unavailable (Friess et al, 1998) or no response was received (Campani et al, 2001).

The five eligible studies included a total of 314 patients with a median number of 63 patients per study (range = 52 to 70) - *Table 5*. Three studies reported a significant prognostic effect of bcl-2 expression on univariate analysis and all studies reported the direction of the survival effect to favour bcl-2 positivity. The median quality score was recorded as 75% (range = 65% to 85%) and the median proportion of bcl-2 positive cases was 33% (range = 12% to 67%).

Meta-analysis

fig.3 illustrates the Forrest plot for the pooled survival data. There was no evidence of any significant heterogeneity ($\chi^2 = 1.19$, $p = 0.88$). The combined HR was recorded as 0.51 (95% CI = 0.38 to 0.68) indicating a significant association between positive bcl-2 immunostaining and more favourable survival in the pooled patient group. Despite the limited number of studies included, the funnel plot for this analysis failed to demonstrate any obvious asymmetry (*fig.4*). Three studies reported use of either adjuvant chemotherapy or chemoradiation and a single study (Bold et al, 1999) also reported use of neoadjuvant chemoradiation in 43 out of the 70 patients analysed. Of the two studies excluded due to incomplete survival data (Friess et al, 1998; Campani et al, 2001), both failed to observe any significant prognostic effect associated with bcl-2 expression.

Table 5 - Methodological and clinico-pathological data for eligible prognostic studies evaluating bcl-2.

	Year	n	HR (95% CI)	Significant	1° Ab (+ dilution)	IHC +ve	IHC cut-off	Male	Age	N1	T3/T4	Well	Mod.	Poor	Adjuvant therapy
bcl-2															
Bold et al	1999	70	0.64 (0.35-1.18)	No	DAKO (NS)	23 (33)	>25%	36 (51)	64	32 (46)	NS	15 (22)	37 (55)	15 (22)	19 (27)
Nio et al	2001	66	0.45 (0.25-0.82)	Yes	DAKO M0887 (1:100)	16 (24)	>5%	31 (47)	66	54 (82)	NS	33 (50)	29 (44)	4 (6)	36 (55)
Magistrelli et al	2002	67	0.56 (0.33-0.96)	Yes	DAKO c124 (1:40)	45 (67)	>5%	45 (67)	63	34 (51)	40 (62)	14 (21)	28 (42)	15 (22)	30 (45)
Sarela et al	2002	52	0.50 (0.08-3.33)	No	DAKO (1:40)	6 (12)	>10%	27 (52)	64	40 (78)	49 (94)	11 (22)	24 (47)	16 (31)	NS
Dong et al	2005	59	0.43 (0.25-0.74)	Yes	DAKO M124(1:100)	21 (36)	>5%	19 (32)	55	54 (82)	NS	19 (32)	21 (36)	19 (32)	NS

% in parentheses unless otherwise stated. NS = not specified. NC = non-commercial.

fig.3 - Forrest plot to assess overall effect of bcl-2 expression on survival.

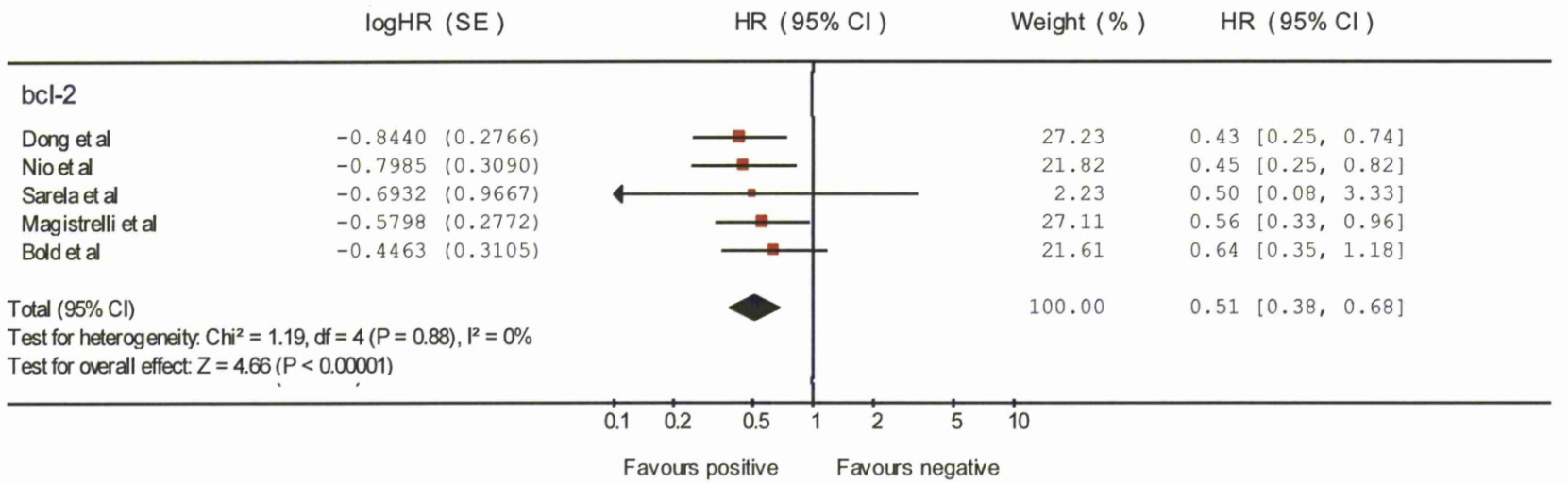
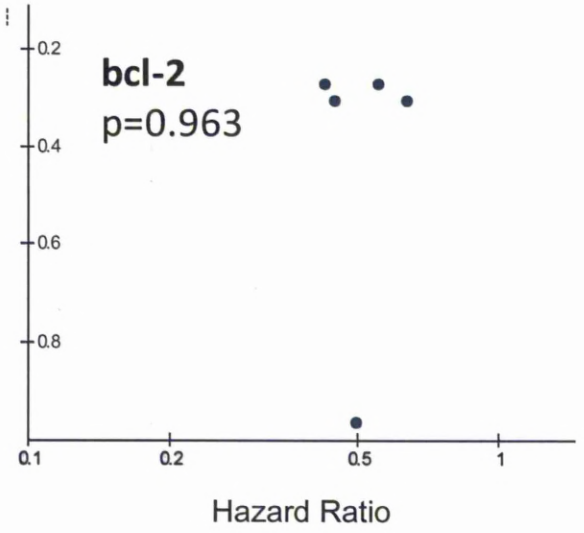


fig.4 - Inverted funnel plot to assess potential publication bias for bcl-2 studies.



3.2.3. bax

Selection Criteria

The following search criteria were used:

- ('bax') AND ('pancreas' OR 'pancreatic') AND ('survival' OR 'prognosis' OR 'prognostic')

Results

The initial search yielded 76 studies. Following review of the abstracts, a total of seven potentially eligible articles were identified. Two of these were excluded due to either a duplicated patient series (Hashimoto et al, 2005) or the inclusion of periampullary cancers of non-pancreatic origin in the survival analysis (Tomazic et al, 2004). Three of the five eligible studies investigated the individual prognostic effect of both bcl-2 and bax and were therefore included in both meta-analyses (Nio et al, 2001; Magistrelli et al, 2002; Dong et al, 2005).

The five eligible studies investigating bax included a total of 274 patients with a median number of 60 patients per study (range = 23 to 67) - *Table 6.* Three of the five studies reported a significant survival advantage associated with bax expression. The median quality score was 65% (range = 55% to 85%) and the median proportion of bax positive cases was 54% (range = 26% to 83%). *fig.5* illustrates the Forrest plot for the pooled survival data. There was no evidence of any significant heterogeneity in the recorded log hazard ratios (χ^2 = 4.25, p = 0.37). The combined hazard ratio was recorded as 0.63 (95% CI = 0.48 to 0.83) and the funnel plot for this analysis is shown in *fig.6.*

Table 6 - Methodological and clinico-pathological data for eligible prognostic studies evaluating bax.

	Year	n	HR (95% CI)	Significant	1° Ab (+ dilution)	IHC +ve	IHC cut-off	Male	Age	N1	T3/T4	Well	Mod.	Poor	Adjuvant therapy
bax															
Friess et al	1998	60	0.47 (0.23-0.97)	Yes	Santa Cruz (NS)	50 (83)	NS	32 (53)	63	38 (63)	NS	NS	NS	NS	NS
Evans et al	2001	23	0.80 (0.28-2.29)	No	Santa Cruz (1:1600)	6 (26)	>5%	15 (65)	59	38 (63)	NS	5 (22)	13 (54)	5 (22)	0 (0)
Nio et al	2001	65	0.49 (0.28-0.85)	Yes	DAKO A3533 (1:100)	42 (65)	>10%	31 (47)	66	54 (82)	NS	33 (50)	29 (44)	4 (6)	36 (55)
Magistrelli et al	2002	67	0.56 (0.33-0.95)	Yes	Zymed c2D2 (1:80)	36 (54)	>10%	45 (67)	63	34 (51)	40 (62)	14 (21)	28 (42)	15 (22)	30 (45)
Dong et al	2005	59	0.93 (0.57-1.52)	No	DAKO A3533 (1:100)	29 (49)	>10%	19 (32)	55	54 (82)	NS	19 (32)	21 (36)	19 (32)	NS

% in parentheses unless otherwise stated. NS = not specified. NC = non-commercial.

fig.5 - Forrest plot to assess overall effect of bax expression on survival.

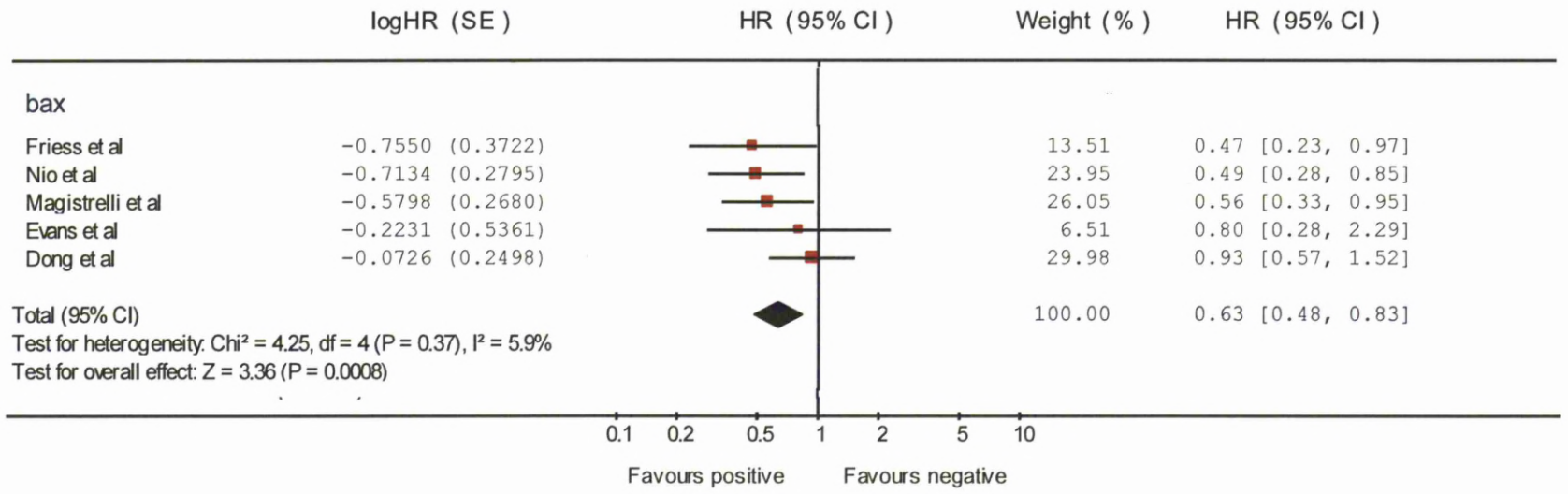
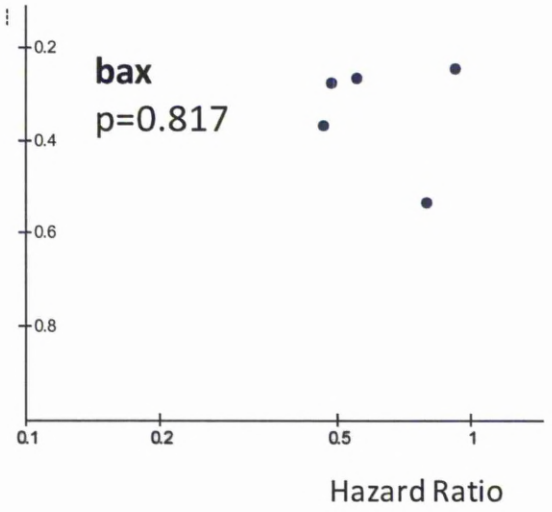


fig.6 - Inverted funnel plot to assess potential publication bias for bax studies.



Discussion

Both bcl-2 and bax emerged as potentially relevant immunohistochemical prognostic factors. These proteins belong to the bcl-2 family and regulate apoptosis by mediating cytosolic release of cytochrome C from mitochondria in response to cellular stress. Cytochrome C binds to APAF-1 and cleaves caspase-9 into its active form, thereby initiating the activation of executioner caspases resulting in cytoskeletal degradation and cell death (Hamacher et al, 2008). The bcl-2-associated X protein (bax) promotes release of cytochrome C and consequently exhibits pro-apoptotic properties. In contrast, bcl-2 inhibits mitochondrial release of cytochrome C and has anti-apoptotic effects as a result. The finding that bax expression is associated with more favourable survival in resected pancreatic cancer is, therefore, concordant with its physiological role. The observation that the same relationship is consistently seen for bcl-2 expression appears paradoxical. However, this finding is mirrored in other malignancies (Martin et al, 2003; Callagy et al, 2008) and it is believed that a complex interaction of competitive dimerisations between pro- and anti-apoptotic proteins governs the cell's fate in response to apoptotic stimuli (Westphal et al, 2003).

All of the eligible bcl-2 studies used commercially available antibodies from the same manufacturer. Despite differences in dilutions and cut-off values used for immunostaining, the five studies returned broadly comparable HR estimates in the same direction. There was more variability in the source of primary antibodies used and the overall range of individual estimated hazard ratios seen in the bax studies. However, where reported, the cut-off values used for IHC scoring were comparable, and the direction of the survival effect of bax expression was consistent across the five eligible studies.

It is difficult to draw any reliable conclusions regarding the prognostic value of bcl-2 and bax expression for resected pancreatic cancer from the current meta-analysis due to the limited number of evaluable studies. Furthermore, data was unobtainable for two relevant bcl-2 studies, both of which recorded no significant association between bcl-2 expression and survival. Despite this, the overall trend towards both bax and bcl-2 expression being associated with more favourable survival outcomes is consistent with the findings reported for other malignancies.

3.2.4. p16

Selection Criteria

The following search criteria were used:

- ('p16' OR 'p16*' OR 'CDKN2A') AND ('pancreas' OR 'pancreatic') AND ('survival' OR 'prognosis' OR 'prognostic')

The initial search returned 91 studies, seven of which were potentially relevant. Following review of these seven articles, three fulfilled all of the eligibility criteria. The remaining studies were rejected due to the inclusion of unresected cases (Hu et al, 1997; Biankin et al,

2002), no IHC used in tissue analysis (Ohtsubo et al, 2003) or only disease-free survival times reported (Jeong et al, 2005). A total of 229 patients were included in the pooled analysis.

Results

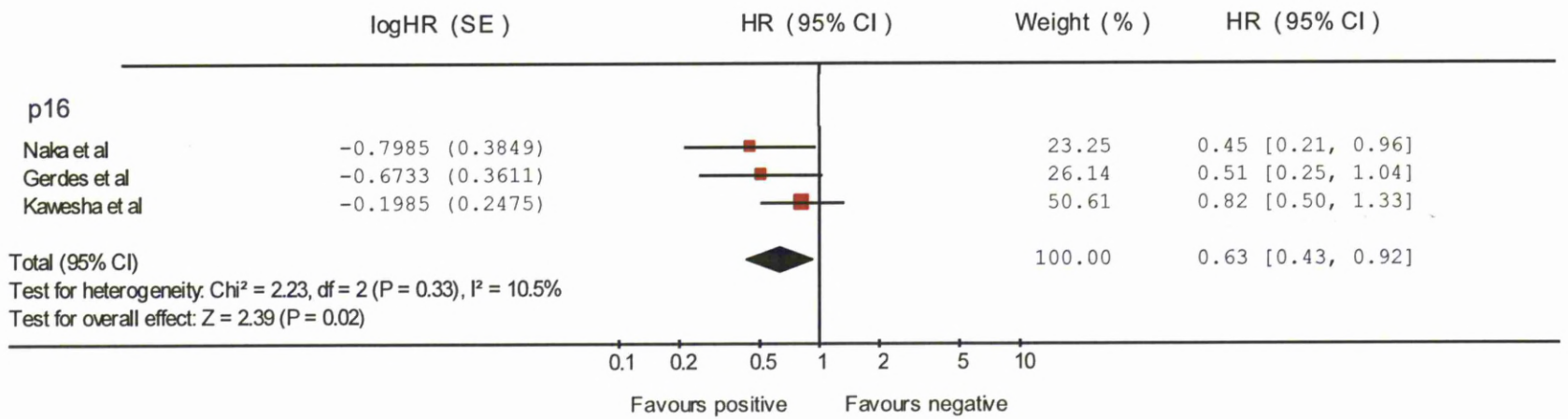
All three studies reported the survival trend to favour positive p16 expression. However, this only reached significance in one (Naka et al, 1998). There was no significant heterogeneity across the three included studies ($\chi^2 = 2.23$, $p = 0.33$). A combined hazard ratio of 0.64 (95% CI = 0.45 to 0.91) was obtained indicating a significant association between p16 expression and more favourable survival in the pooled patient group. No analysis of the funnel plot was undertaken due to the limited number of eligible studies.

Table 7 - Methodological and clinico-pathological data for eligible prognostic studies evaluating p16.

	Year	n	HR (95% CI)	Significant	1° Ab (+ dilution)	IHC +ve	IHC cut-off	Male	Age	N1	T3/T4	Well	Mod.	Poor	Adjuvant therapy
p16															
Naka et al	1998	32	0.45 (0.21-0.96)	Yes	Santa Cruz C20 (1:500)	19 (59)	NS	20 (63)	65	23 (72)	13 (41)	NS	NS	NS	NS
Kawesha et al	2000	157	0.82 (0.50-1.33)	No	Santa Cruz (1:100)	21 (13)	>5%	100 (64)	60	71 (46)	NS	21 (13)	77 (49)	59 (38)	13 (8)
Gerdes et al	2002	40	0.51 (0.25-1.04)	No	Pharmingen G175-405 (1:50)	13 (33)	>5%	22 (55)	NS	16 (40)	NS	NS	NS	NS	0 (0)

% in parentheses unless otherwise stated. NS = not specified. NC = non-commercial.

fig. 7 - Forrest plot to assess overall survival effect of p16 expression.



Discussion

The tumour suppressor gene p16 (CDKN2A) plays a key role in pancreatic carcinogenesis (Schutte et al, 1997). p16 is a cell-cycle checkpoint protein which binds to cyclin-dependent kinases resulting in cell cycle arrest at the G1/S checkpoint. The observation that positive immunostaining for p16 appears to represent a favourable prognostic feature is, therefore, also consistent with its tumour suppressor function. Despite this, the three studies reported very disparate proportions of IHC positivity ranging from 13% to 59% and the small number of eligible studies included in this analysis again precludes any meaningful conclusions being drawn regarding the reproducibility of p16 expression as a reliable marker of prognosis in resected pancreatic cancer.

3.2.5. p53

Selection Criteria

The following search criteria were used:

- ('p53' OR 'TP53') AND ('pancreas' OR 'pancreatic') AND ('survival' OR 'prognosis' OR 'prognostic')

The initial search returned a total of 337 studies. Following review of these abstracts, 58 potentially relevant studies were retrieved of which 17 fulfilled all of the inclusion criteria. The remaining studies were rejected for the following reasons: duplicated series of patients (Dergham et al, 1997a; Nio et al, 1998; Dong et al, 1998; Nio et al, 1999; Dong et al, 2000; Nio et al, 2001; Linder et al, 2001), no dichotomised univariate survival analysis conducted (Sessa et al, 1998; Karademir et al, 2000; Fujioka et al, 2001; Campani et al, 2001; Evans et al, 2001; Gazzaniga et al, 2001; Biankin et al, 2002; Dang et al, 2002; Hashimoto et al, 2005; Smeenk et al, 2007; Dong et al, 2007) , no IHC used in tissue analysis (Weyrer et al, 1996; Li

et al, 1999; Yamaguchi et al, 2000; Ohshio et al, 2002; Dong et al, 2003), unresected cases included in survival analysis (Zhang et al, 1994; Aizawa et al, 1996; Lundin et al, 1996; Coppola et al, 1998; Dergham et al, 1998; Mäkinen et al, 1998; Ohshio et al, 1998; Hu et al, 1999); Takikita et al, 2009), mix of different tumour types included (Sinicrope et al, 1996; Sato et al, 1997; Gansauge et al, 1998; Yu et al, 2004) , only disease-free survival reported (Jeong et al, 2005), insufficient survival data reported (Dergham et al, 1997b; Campani et al, 1999; Stipa et al, 2002; Hermanova et al, 2009). The four corresponding authors were contacted for the potentially eligible studies where insufficient survival data were reported. Supplementary data were either unavailable (Dergham et al, 1997b) or no response was received (Campani et al, 1999; Stipa et al, 2002; Hermanova et al, 2009).

The 17 eligible studies included a total of 925 patients with a median number of 48 patients per study (range = 26 to 157) - *Table 8*. Nuclear staining of p53 was used for scoring in all cases. Five studies (29%) reported a significant adverse association between p53 expression and survival. The median quality score was recorded as 65% (range = 45% to 90%) and the median proportion of patients exhibiting positive p53 immunostaining was 47% (range = 25% to 68%). There was no significant association between the IHC cut-off score used and the proportion of cases classified as p53 positive (Spearman, rho = 0.389, p = 0.206). Furthermore, there was no significant difference in median quality scores between significant and non-significant studies (Mann-Whitney, p = 0.243).

fig.8 illustrates the Forrest plot for the survival data. There was no evidence of any significant publication bias (Egger's test, p = 0.298). However, significant heterogeneity was demonstrated (χ^2 = 37.88, p = 0.002). The combined HR was recorded as 1.22 (95% CI = 0.96 to 1.56) indicating no significant overall association between p53 expression and

survival. Of the three studies excluded due to incomplete reporting of survival data, only one reported a significant association between p53 expression and survival (Stipa et al, 2002).

Table 8 - Methodological and clinico-pathological data for eligible prognostic studies evaluating p53.

	Year	n	HR (95% CI)	Significant	1° Ab (+ dilution)	IHC +ve	IHC cut-off	Male	Age	N1	T3/T4	Well	Mod.	Poor	Adjuvant therapy
p53															
DiGiuseppe et al	1994	48	1.77 (0.95-3.29)	No	Novocastra CM-1 (1:1000)	26 (54)	NS	25 (52)	61	NS	NS	NS	NS	NS	NS
Yokoyama et al	1994	57	1.75 (1.01-3.02)	Yes	Novocastra DO7 (1:100)	33 (58)	NS	NS	64	25 (45)	27 (47)	37 (65)		20 (35)	NS
Lee et al	1995	26	1.17 (0.43-3.16)	No	Biogenex CM1 (NS)	7 (27)	NS	14 (54)	NS	NS	NS	2 (8)	20 (77)	4 (15)	NS
Linder et al	1997	48	2.71 (1.41-5.20)	Yes	DAKO DO7 (1:50)	22 (46)	>1%	36 (68)	66	18 (38)	26 (49)	5 (9)	18 (34)	30 (57)	NS
Virkajarvi et al	1997	36	0.99 (0.46-2.11)	No	Novocastra CM-1 (1:1000)	15 (42)	>1%	16 (44)	64	NS	NS	NS	NS	NS	NS
Naka et al	1998	32	0.84 (0.34-2.10)	No	Novocastra BP53-12 (1:50)	19 (59)	NS	20 (63)	65	23 (72)	13 (41)	NS	NS	NS	NS
Bold et al	1999	70	0.62 (0.34-1.12)	No	Oncogene DO1 (NS)	33 (47)	>25%	36 (51)	64	38 (54)	NS	15 (22)	37 (56)	15 (22)	19 (27)
Gansauge et al	1999	26	2.97 (1.30-6.79)	Yes	Oncogene DO1 (1:500)	11 (42)	NS	12 (50)	59	22 (85)	NS	NS	NS	NS	26 (100)
Ahrendt et al	2000	43	0.77 (0.39-1.52)	No	DAKO DO7 (1:2000)	26 (60)	>33%	24 (55)	63	23 (53)	22 (51)	11 (26)	23 (55)	8 (19)	29 (66)
Bergan et al	2000	60	2.16 (1.14-4.08)	Yes	Novocastra DO7 (1:100)	15 (25)	>5%	41 (50)	62	18 (30)	21 (35)	25 (42)	23 (38)	12 (20)	0 (0)
Kawesha et al	2000	157	0.90 (0.64-1.26)	No	DAKO DO7 (1:300)	64 (41)	>5%	100 (64)	60	71 (46)	NS	21 (13)	77 (49)	59 (38)	13 (8)
Gerdes et al	2002	40	1.16 (0.61-2.21)	No	DAKO DO7 (1:400)	13 (33)	>10%	22 (55)	NS	16 (40)	NS	NS	NS	NS	0 (0)
Sarela et al	2002	52	0.53 (0.11-2.53)	No	DAKO DO7 (1:100)	28 (54)	>10%	27 (52)	64	40 (78)	49 (94)	11 (22)	24 (47)	16 (31)	NS
Yamasawa et al	2002	72	1.01 (0.62-1.66)	No	Oncogene DO1 (2µg/ml)	34 (47)	>20%	34 (47)	65	21 (29)	42 (58)	35 (49)	32 (44)	5 (7)	41 (57)
Dong et al	2005	59	1.02 (0.60-1.73)	No	DAKO DO7 (1:20)	40 (68)	>10%	38 (64)	NS	47 (80)	NS	19 (32)	21 (36)	19 (32)	NS
Magistrelli et al	2006	67	0.68 (0.40-1.16)	No	DAKO DO7 (1:50)	32 (48)	>5%	45 (67)	63	34 (51)	40 (62)	14 (21)	28 (42)	15 (22)	30 (45)
Jinfeng et al	2007	32	3.18 (1.30-7.77)	Yes	DAKO DO7 (1:50)	13 (41)	>10%	19 (59)	63	18 (56)	23 (72)	11 (34)	18 (56)	3 (10)	NS

% in parentheses unless otherwise stated. NS = not specified. NC = non-commercial.

fig.8 - Forrest plot for pooled data from eligible studies evaluating p53.

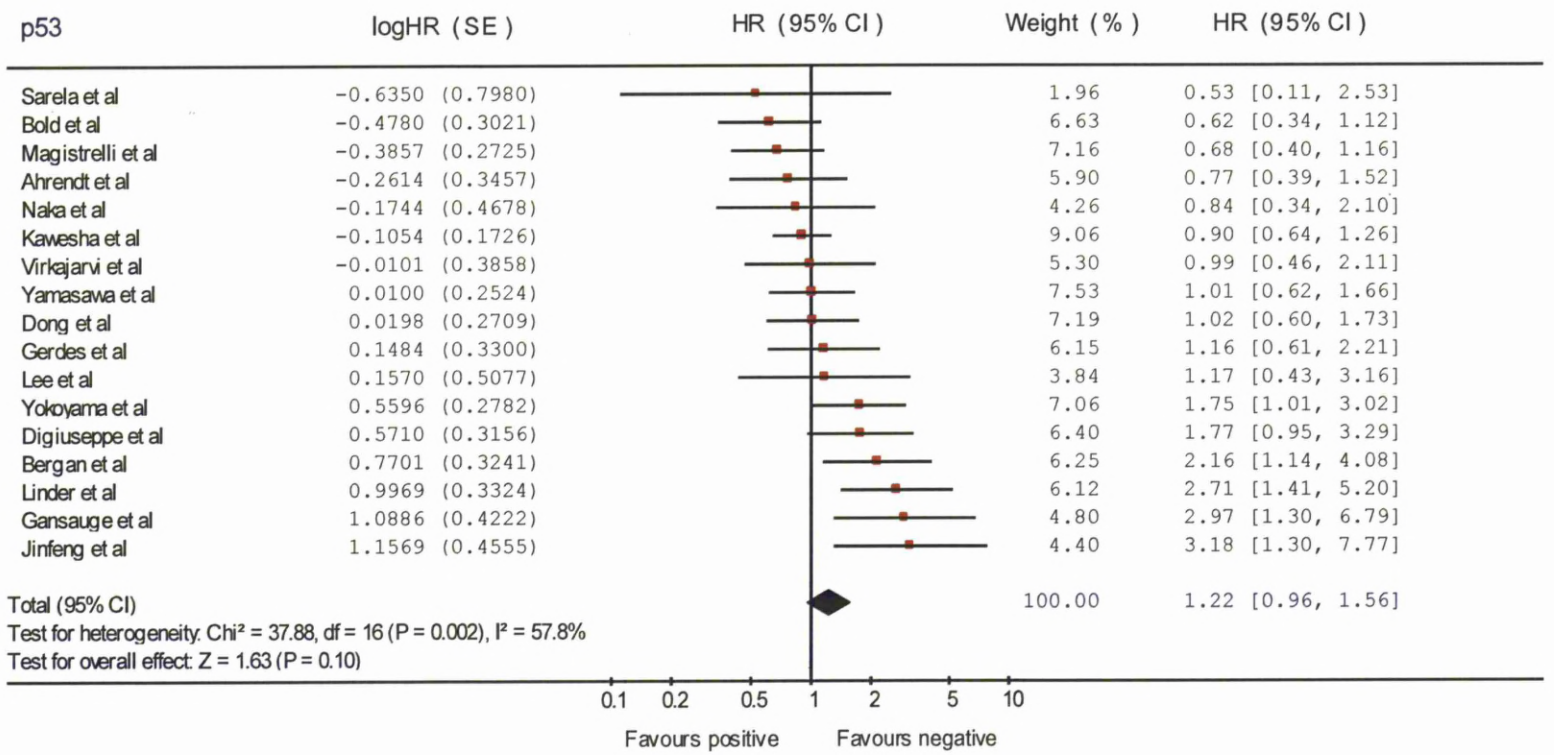
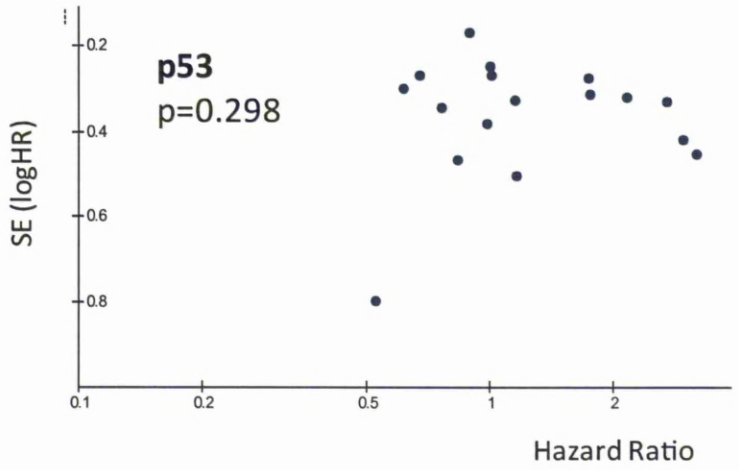


fig.9 - Funnel plot for pooled survival data from evaluable p53 studies.



Discussion

The tumour suppressor protein p53 represents the most extensively investigated immunohistochemical prognostic marker of those selected for analysis as part of this study. It was also found to exhibit the greatest degree of heterogeneity in the reported association between immunostaining and survival for individual studies. Although the overall trend was towards overexpression of p53 resulting in adverse survival for the pooled data, this did not reach significance and there is no obvious explanation for the contradictory results seen between the various studies. The majority of studies used either the monoclonal DO-7, DO-1 or polyclonal CM-1 primary antibodies which all exhibit similar immunoreactivity with both wild-type and mutant forms of p53. Due to the increased stability of mutant p53, most of the nuclear immunostaining seen reflects the presence of the mutant rather than wild-type p53 protein.

Despite the marked differences between studies in terms of the proportion of cases classified as p53 positive, the reported primary antibody dilutions used and the various cut-off values selected for immunohistochemical scoring, there was no clear association between any of

these factors and either the direction of the prognostic effect or the reported magnitude of the hazard ratio which might potentially explain the disparity in survival trends. These results indicate that p53 expression in primary tumour material fails to represent a reliable or reproducible prognostic factor in patients with resected pancreatic cancer.

When considering the question of publication bias for the p53 literature, only five of the 17 evaluable studies reported a significant relationship between p53 immunostaining and survival. All of these five significant studies reported the direction of the effect to reflect adverse survival associated with positive staining. Furthermore, the funnel plot failed to demonstrate any evidence of marked asymmetry and Egger's regression for this dataset returned a non-significant p-value. Only one of the three studies omitted due to incomplete reporting of survival data demonstrated a significant relationship between p53 and survival. These results indicate that publication bias is unlikely to represent a significant confounding factor in the interpretation of these results.

3.2.6. smad4

Selection Criteria

The following search criteria were used:

- ('smad4' OR 'smad-4' OR 'smad*' OR 'DPC4' OR 'DPC-4' OR 'DPC*') AND ('pancreas' OR 'pancreatic') AND ('survival' OR 'prognosis' OR 'prognostic')

The initial search returned 81 studies. Following review of these abstracts, five potentially relevant studies were identified which were all found to be eligible for analysis. The combined number of patients was 540 with a median of 88 patients per study (range = 34 to 249) - *Table 9*. Three studies reported a significant relationship between smad4 expression

and survival. A single study (Biankin et al, 2002) reported the direction of the survival effect to favour loss of smad4 expression.

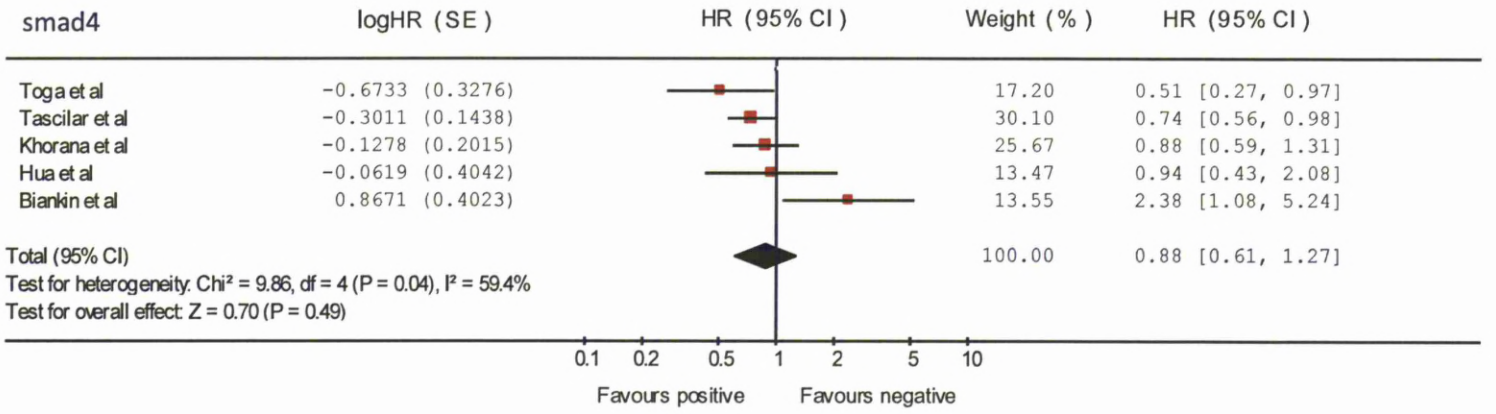
The median quality score was 75% (range = 60% to 95%) and the median proportion of patients exhibiting positive smad4 immunostaining was 45% (range = 15% to 76%). *fig.10* illustrates the Forrest plot. Significant heterogeneity was demonstrated between the included studies ($\chi^2 = 9.86$, $p = 0.04$). A combined HR of 0.88 (95% CI = 0.61 to 1.27) was recorded indicating no significant overall association between smad4 expression and survival in the pooled patient group.

Table 9 - Methodological and clinico-pathological data for eligible prognostic studies evaluating p53.

	Year	n	HR (95% CI)	Significant	1° Ab (+ dilution)	IHC +ve	IHC cut-off	Male	Age	N1	T3/T4	Well	Mod.	Poor	Adjuvant therapy
smad4															
Tascilar et al	2001	249	0.74 (0.56-0.98)	Yes	Santa Cruz B8 (1:100)	111 (46)	NS	139 (56)	65	NS	NS	NS	NS	NS	Yes (n=?)
Biankin et al	2002	45	2.38 (1.08-5.24)	Yes	Santa Cruz B8 (NS)	10 (22)	>5%	27 (60)	61	21 (47)	NS	5 (11)	28 (62)	12 (27)	8 (16)
Hua et al	2003	34	0.94 (0.43-2.08)	No	Santa Cruz B8 (1:100)	26 (76)	NS	22 (65)	55	14 (41)	NS	27 (79)		7 (21)	NS
Toga et al	2004	88	0.51 (0.27-0.97)	Yes	Santa Cruz B8 (1:100)	13 (15)	>10%	43 (49)	66	78 (89)	33 (37)	37 (42)	45 (51)	6 (7)	58 (66)
Khorana et al	2005	124	0.88 (0.59-1.31)	No	Santa Cruz (1:400)	59 (48)	>5%	69 (56)	67	56 (45)	69 (58)	23 (19)	52 (43)	45 (38)	88 (79)

% in parentheses unless otherwise stated. NS = not specified. NC = non-commercial.

fig.10 - Forrest plot for pooled survival data from evaluable smad4 studies.



Discussion

The smad4 (or DPC4) protein is a central component of the intracellular signalling pathway for transforming growth factor β (TGF-β) and loss of smad4 expression represents an important event in the progression of PanINs to invasive malignancy (Wilentz et al, 2000). The results from the analysis of the five studies evaluating smad4 expression demonstrate unexplained heterogeneity in the reporting of the prognostic effect of this marker. Biankin et al reported an entirely contradictory survival trend to the other four studies with loss of smad4 expression being associated with significantly *improved* survival in their patient group despite use of the same primary antibody and otherwise broadly comparable study methodology and clinico-pathological characteristics. This survival trend appears at odds with the accepted tumour suppressor role of smad4 in mediating the inhibitory signalling associated with the TGF-β pathway.

Despite the fact that the patient series reported by Biankin et al only accounts for 8% of all patients included in the combined analysis and 14% of the weighting allocated to the pooled survival data, the discrepancy in the results is such that sufficient heterogeneity is introduced to result in a non-significant result for the overall analysis when using a random effects approach. These findings further underline the difficulties in making any reliable conclusions regarding the relative prognostic value of immunohistochemical markers when analysed in limited patient series.

3.2.7. EGFR

Selection Criteria

The following search criteria were used:

- ('epidermal growth factor receptor' OR 'EGFR' OR 'c-erbB*' OR 'erbB*' OR 'HER*') AND ('pancreas' OR 'pancreatic') AND ('survival' OR 'prognosis' OR 'prognostic')

The initial search identified 324 studies. Following review of these abstracts, ten potentially relevant articles were retrieved. Six of these were studies were rejected for the following reasons: duplicated series of patients (Ueda et al, 2006; Uegaki et al, 1997), no dichotomised univariate survival analysis conducted (Yamanaka et al, 1993; Zhang et al, 2002), unresected cases included in analysis (Gansauge et al, 1998; Takikita et al, 2009).

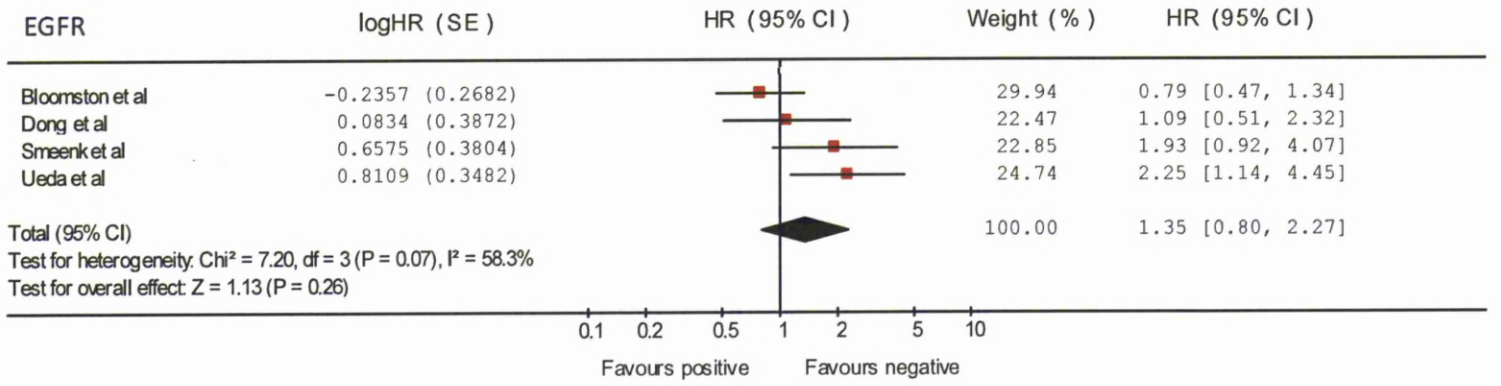
The four eligible studies included a total of 250 patients (*Table 10*). Only a single study reported a significant relationship between EGFR expression and survival (Ueda et al, 2004). The median quality score was 70% (range = 65% to 70%). *fig.11* illustrates the Forrest plot for the pooled data. Significant heterogeneity was demonstrated on Cochran's χ^2 test (χ^2 = 7.20, p = 0.07). The combined HR was recorded as 1.35 (95% CI = 0.80 to 2.27) indicating no significant overall association between EGFR expression and survival.

Table 10 - Methodological and clinico-pathological data for eligible prognostic studies evaluating EGFR.

	Year	n	HR (95% CI)	Significant	1° Ab (+ dilution)	IHC +ve	IHC cut-off	Male	Age	N1	T3/T4	Well	Mod.	Poor	Adjuvant therapy
EGFR															
Dong et al	1998	57	1.09 (0.51-2.32)	No	Oncogene 985/996 (1:20)	39 (68)	NS	20 (35)	55	46 (81)	NS	18 (32)	22 (39)	17 (30)	7 (12)
Ueda et al	2004	76	2.25 (1.14-4.45)	Yes	Zymed 31G7 (1:200)	47 (62)	>10%	57 (75)	63	59 (78)	NS	11 (14)	32 (42)	33 (43)	NS
Bloomston et al	2006	71	0.79 (0.47-1.34)	No	Dakocytomation 218C9 (NS)	49 (69)	>1%	40 (56)	65	41 (58)	57 (81)	6 (9)	45 (63)	20 (28)	NS
Smeenk et al	2007	46	1.93 (0.92-4.07)	No	DAKO H11 (NS)	11 (24)	>1%	37 (66)	63	29 (52)	34 (61)	6 (11)	43 (77)	7 (12)	19 (34)

% in parentheses unless otherwise stated. NS = not specified. NC = non-commercial.

fig.11 - Forrest plot to assess overall survival effect of EGFR.



Discussion

Epidermal growth factor receptor (EGFR) is the cell surface receptor for a family of extracellular ligands which include EGF and TGF-α and is coded for by the c-erbB1 proto-oncogene. Activation of EGFR stimulates intracellular tyrosine kinase phosphorylation with consequent activation of a number of signalling cascades including the MAPK (mitogen-activated protein kinase) and Akt (protein kinase) pathways which promote cell proliferation (Ciardello et al, 2008).

The analysis of the four eligible studies included in the current meta-analysis again fails to make a strong case for tumoral over-expression of EGFR representing a reproducible prognostic marker. However, the laboratory methodologies reported in the four studies demonstrated more marked variability (eg. use of four different EGFR primary antibodies) when compared with some of the other analyses.

4. OVERALL CONCLUSIONS & FUTURE WORK

The results from Chapter 2 demonstrate the expected relationships between resected histological tumour characteristics and postoperative survival in the cohort of pancreatic cancer resections analysed. Tumour size, differentiation and nodal status emerged as the most important histological prognostic factors. These results provide further evidence to indicate that the lymph node ratio represents a more informative prognostic factor when compared with overall nodal status. The analysis of resection margin status provides the first clinical evidence to support previous Royal College of Pathologists recommendations regarding resection margin reporting in pancreatoduodenectomy cancer specimens and indicates that pathological reporting criteria have a major impact on R1 resection rates. These findings have important implications for multicentre trials when evaluating risk stratification according to resection margin status.

Elevated preoperative serum CA19-9 levels were shown to be a determinant of adverse postoperative survival which also demonstrate a significant relationship with microscopic tumour burden. The platelet-lymphocyte ratio represents a newly described prognostic factor which exhibits a strong association with both tumour resectability, likelihood of patient selection for adjuvant therapy and overall survival following resection. This index has also been shown to have potential prognostic value in resected ampullary cancer (Smith et al, 2008) in addition to other tumour types (Aliustaoglu et al, 2009). The potential importance of the preoperative inflammatory response in the study group is also evident from the prognostic relationships described for both C-reactive protein and albumin. These findings have influenced the prospective data collected as part of the ESPAC-4 trial which will allow future study of these prognostic indices in a much larger multicentre cohort of patients.

The analysis of the combined preoperative score suggests that comparable prognostic information can be derived from routine preoperative haematological and biochemical data as from the histopathology report for the resected tumour specimen. If validated in larger future studies, these results may be of clinical utility in identifying a sub-group of patients with potentially resectable pancreatic malignancy who may not benefit from surgical resection.

The findings from this study have prompted further work investigating the prognostic value of various circulating cytokines in archived serum collected from patients undergoing pancreatic cancer resections. This study will also investigate the relationship between these inflammatory mediators and the preoperative haematological and biochemical parameters described in the present study.

Meta-analysis of prognostic literature is associated with a number of inherent limitations. One of the key limitations is the general prevalence of retrospective study design in this setting. None of the studies included in the current meta-analysis specified a prospective design and archived paraffin-embedded tumour material was utilised for IHC in all cases. This indicates that availability of tissue is invariably the main determinant of study size rather than any specific considerations relating to adequate statistical power in order to reliably detect a prognostic effect for the biomarker of interest. The availability and adequacy of corresponding clinico-pathological data is also a significant consideration in retrospective studies of this type and we identified several studies reporting incomplete datasets with regard to histopathological details. Alongside this, an additional hindrance to meta-analysis of prognostic literature is the general lack of multivariable survival data. This is usually attributable to the fact that the number of patients included in each study is typically small, precluding any meaningful attempt at analysing multiple covariates.

Additional challenges in the interpretation and comparison of immunohistochemical prognostic studies include variability in patient selection (ie. resected and unresected cases, inclusion of non-pancreatic periampullary tumours), disparate immunohistochemical criteria used for prognostic classification, potential bias associated with the statistical approach to analysis of survival data (eg. selection of data-driven cut-off values for continuous variables), incomplete reporting of survival data, duplicated patient series and publication bias arising as a result of selective reporting of 'positive' studies (Altman, 2001). In order to overcome some of these comparative difficulties, specific inclusion criteria were applied in order to select literature for meta-analysis. Only studies including resected pancreatic adenocarcinoma were included in order to avoid any confounding effects on survival associated with differing proportions of resected and unresected cases. Any studies including periampullary tumours of non-pancreatic origin were also excluded due to the disparity in survival outcomes characteristically associated with ampullary, duodenal and bile duct adenocarcinomas when compared with pancreatic adenocarcinoma (Riall et al, 2006). Furthermore, in cases where part or all of the same patient series was included in more than one publication, only the more recent or most complete study was included in the analysis in order to avoid duplicating the same patient data for the immunohistochemical marker of interest. For those studies where insufficient survival data were reported to generate indirect calculations for the logHR and variance, authors were contacted for additional survival data. However, in all cases the authors were either unable to provide any supplementary data or no response was received. The only supplementary raw data obtained was for two studies previously conducted at our own institution (Kawesha et al, 2000; Evans et al, 2001). Therefore, no subsequent attempt to request individual patient survival data for all eligible studies was undertaken, although this would have been potentially beneficial.

When analysing the overall relationships between individual study size, reported prognostic significance and methodological quality scores in the present study, there was a significant trend towards superior methodological quality in larger studies as one might reasonably expect, despite the fact that study size itself was not one of the criteria used for quality scoring. When considering the overall effect of potential publication bias in this analysis, only a minority of studies (21 out of 50) actually reported a statistically significant prognostic result. Furthermore, the funnel plots and Egger's tests for the individual analyses, although more difficult to interpret when fewer studies were included, were not generally indicative of any strong publication bias.

Vascular endothelial growth factor (VEGF) emerged as the most potentially informative prognostic factor when analysing the pooled survival data from all of the molecular markers included in this meta-analysis. The direction and order of magnitude for the observed prognostic effect of VEGF immunostaining in resected pancreatic cancer is in keeping with results from previously conducted meta-analyses investigating the prognostic role of VEGF immunostaining for other malignancies (Des Guetz et al, 2006; Delmotte et al, 2002). The observed association between VEGF staining and T stage when pooling the histological data is also in concordance with the biological role of VEGF. Analysis of the funnel plot suggests that publication bias is unlikely to represent a significant confounding effect when evaluating these results.

The results for *bcl-2*, *bax* and p16 all indicated a significant overall prognostic effect for each molecular marker. However, given the smaller numbers of studies included in each analysis, these findings should be interpreted interpreted with caution. Despite representing the most widely investigated molecular prognostic marker for resected pancreatic cancer, the pooled

survival data fails to suggest that p53 immunostaining has any prognostic significance in this setting. This was similarly true for smad4 and EGRF indicating that these molecular markers are of less prognostic value in resected pancreatic cancer.

These findings are of relevance for future research given the increasing potential for use of adjuvant therapies tailored to patient-specific tumour biology (Farrell et al, 2009). The results indicate that future studies utilising microarrayed tissue should consider inclusion of VEGF as a comparative molecular marker of prognosis in patients with resected pancreatic cancer. These results provide further evidence to suggest that in order to make reliable conclusions regarding immunohistochemical prognostic factors and to identify the relevance with which these factors can be translated into clinical use (eg. individualised patient selection for adjuvant therapy modalities), large collaborative studies utilising microarrayed tissue as part of prospective multicentre trials, with standardised approaches to both laboratory and statistical methodology, represent the optimal strategy to achieve these goals in the future (eg. Farrell et al, 2008; Manuyakorn et al, 2010). The findings of this meta-analysis have been published in 2011 (*Appendix B*).

REFERENCES

Achen MG, Stacker SA. Molecular control of lymphatic metastasis. Ann N Y Acad Sci 2008; 1131:225-234.

Adsay NV, Merati K, Andea A et al. Pathogenesis of colloid (pure mucinous) carcinoma of exocrine organs: coupling of gel-forming mucin (MUC2) production with altered cell polarity and abnormal cell-stroma interaction may be the key factor in the morphogenesis and indolent behaviour of colloid carcinoma in the breast and pancreas. Am J Surg Pathol 2003; 27:571-578.

Ahrendt SA, Brown HM, Komorowski RA et al. p21WAF1 expression is associated with improved survival after adjuvant chemoradiation for pancreatic cancer. Surgery 2000; 128:520-530.

Ai KX, Lu LY, Huang XY et al. Prognostic significance of S100A4 and vascular endothelial growth factor expression in pancreatic cancer. World J Gastroenterol 2008; 14:1931-1935.

Aizawa S, Sasaki M, Wada R et al. P53 protein expression in pancreatic tumors and its relationship to clinicopathological factors and prognosis. J Surg Oncol 1996; 62:279-283.

Alexandrakis MG, Passam FH, Moschandrea IA et al. Levels of serum cytokines and acute phase proteins in patients with essential and cancer-related thrombocytosis. Am J Clin Oncol 2003; 26:135-140.

Aliustaoglu M, Bilici A, Ustaalioglu BB et al. The effect of peripheral blood values on prognosis of patients with locally advanced gastric cancer before treatment. Med Oncol 2009; 22 (Epub).

Altman DG, Lausen B, Sauerbrei W et al. Dangers of using optimal cutpoints in the evaluation of prognostic factors. J Nat Cancer Inst 1994; 86:829-835.

Altman DG. Systematic reviews of evaluations of prognostic variables. BMJ 2001; 323:224-228.

Al-Wadei HA, Schuller HM. Nicotinic receptor-associated modulation of stimulatory and inhibitory neurotransmitters in NNK-induced adenocarcinoma of the lungs and pancreas. J Pathol 2009; 218:437-445.

Al-Wadei HA, Al-Wadei MH, Schuller HM. Prevention of pancreatic cancer by the beta-blocker propanolol. Anticancer Drugs 2009; 20:477-482.

Anderson KE, Sinha R, Kulldorff M et al. Meat intake and cooking techniques: associations with pancreatic cancer. Mutat Res 2002; 506-7:225-231.

Anderson KE, Johnson TW, Lazovich D et al. Association between nonsteroidal anti-inflammatory drug use and the incidence of pancreatic cancer. J Natl Cancer Inst 2002a; 94:1168-1171.

Askari MD, Tsao MS, Schuller HM. The tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone stimulates proliferation of immortalised human pancreatic duct epithelia through beta-adrenergic transactivation of EGF receptors. J Cancer Res Clin Oncol 2005; 131:639-648.

Bakkevold KE, Arnesjo B, Dahl O et al. Adjuvant combination chemotherapy (AMF) following radical resection of carcinoma of the pancreas and papilla of Vater - results of a controlled, prospective, randomised multicentre study. Eur J Cancer 1993; 29:698-703.

Bartsch H, Malaveille C, Lowenfels AB et al. Genetic polymorphism of N-acetyltransferases, glutathione S-transferase MI and NAD(P)H:quinine oxidoreductase in relation to malignant and benign pancreatic disease risk. The International Pancreaic Disease Study Group. Eur J Cancer Prev 1998; 7:215-223.

Bassi C, Stocken DD, Olah A et al. Influence of surgical resection and post-operative complications on survival following adjuvant treatment for pancreatic cancer in the ESPAC-1 randomized controlled trial. Dig Surg 2005; **22:**353-363.

Bellone G, Turletti A, Artusio E et al. Tumor-associated transforming growth factor-beta and interleukin-10 contribute to a systemic Th2 immune phenotype in pancreatic carcinoma patients. Am J Pathol 1999; 155:537-547.

Bellone G, Smirne C, Angelo F et al. Cytokine expression in human pancreatic carcinoma cells in surgical specimens: implications for survival. Cancer Immunol Immunother 2006; 55:684-698.

Benassi G, Mastrorilli M, Quarto G et al. Factors influencing survival after resection for ductal adenocarcinoma of the head of pancreas. J Surg Oncol 2000; **73:**212-218.

Bergan A, Gladhaug IP, Schjolberg A et al. p53 accumulation confers prognostic information in resectable adenocarcinomas with ductal but not with intestinal differentiation in the pancreatic head. Int J Oncol 2000; 17:921-926.

Berger AC, Sigurdson ER, Le Voyer TE et al. Colon cancer survival is associated with decreasing ratio of metastatic to examined lymph nodes. J Clin Oncol 2005; 23:8706-8712.

Biankin AV, Morey AL, Lee CS et al. DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. J Clin Oncol 2002; 20:4531-4542.

Bloomston M, Bhardwaj A, Ellison EC et al. Epidermal growth factor receptor expression in pancreatic carcinoma using tissue microarray technique. Dig Surg 2006; 23:74-79.

Bogoevski D, Yekebas EF, Schurr, Kaifi JT, Kutup A, Erbersdobler *et al.* Mode of spread in the early phase of lymphatic metasis in pancreatic ductal adenocarcinoma: prognostic significance of nodal microinvolvement. Ann Surg 2004; **240:**993-1000.

Bold RJ, Charnsangavej C, Cleary KR et al. Major vascular resection as part of pancreaticoduodenectomy for cancer: radiologic, intraoperative, and pathologic analysis. J Gastrointest Surg 1999; 3:233-143.

Bold RJ, Hess KR, Pearson AS et al. Prognostic factors in resectable pancreatic cancer: p53 and bcl-2. J Gastrointest Surg 1999; 3:263-277.

Bonovas S, Filioussi K, Sitaras NM. Statins are not associated with a reduced risk of pancreatic cancer at the population level when taken at low doses for managing hypercholesterolaemia: evidence from a meta-analysis of 12 studies. Am J Gastroenterol 2008; 103:2646-2651.

Brockmann J, Emparan C, Hernandez CA et al. Gallbladder bile tumor marker quantification for detection of pancreato-biliary malignancies. Anticancer Res 2000; **20:**4941-4947.

Brooks AD, Mallis MJ, Brennan MF et al. The value of laparoscopy in the management of ampullary, duodenal and distal bile duct tumours. J Gastrointest Surg 2002; 6:139-146.

Brown KM, Domin C, Aranha GV et al. Increased preoperative platelet count is associated with decreased survival after resection for adenocarcinoma of the pancreas. Am J Surg 2005; 189:278-282.

Büchler P, Reber HA, Büchler MW et al. VEGF-RII influences the prognosis of pancreatic cancer. Ann Surg 2002; 236:738-749.

Butturini G, Stocken DD, Wente MN et al. Influence of resection margins and treatment on survival in patients with pancreatic cancer: meta-analysis of randomized controlled trials. Arch Surg 2008; 143:75-83.

Callagy GM, Webber MJ, Pharoah PD et al. Meta-analysis confirms bcl-2 is an independent prognostic marker in breast cancer. BMC Cancer 2008; 8:153.

Campani D, Boggi U, Cecchetti D et al. p53 overexpression in lymph node metastases predicts clinical outcome in ductal pancreatic cancer. Pancreas 1999; 19:26-32.

Campani D, Esposito I, Boggi U et al. Bcl-2 expression in pancreas development and pancreatic cancer progression. J Pathol 2001; 194:444-450.

Campbell F, Bennett M, Foulis AJ. *Minimum Dataset for Histopathological Reporting of Pancreatic, Ampulla of Vater and Bile Duct Carcinoma.* Royal College of Pathologists; 2002. http://www.rcpath.org.

Cancer Research UK. http://info.cancerresearchuk.org/ (cited Aug 2007)

Canna K, McArdle PA, McMillan DC et al. The relationship between tumour T-lymphocyte infiltration, the systemic inflammatory response and survival in patients undergoing curative resection for colorectal cancer. Br J Cancer 2005; 92:651-654.

Chung GG, Yoon HH, Zerkowski MP et al. Vascular endothelial growth factor, FLT-1, and FLK-1 analysis in a pancreatic cancer tissue microarray. Cancer 2006; 106:1677-1684.

Ciardello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med 2008; 358:1160-1174.

Coogan PF, Rosenberg L, Palmer JR et al. Nonsteroidal anti-inflammatory drugs and risk of digestive cancers at sites other than the large bowel. Cancer Epidemiol Biomarkers Prev 2000; 9:119-123.

Connor S, Bosonnet L, Ghaneh P, et al. Survival of patients with periampullary carcinoma is predicted by lymph node 8a but not by lymph node 16b1 status. Br J Surg 2004; 91:1592-9.

Connor S, Bosonnet L, Alexakis N et al. Serum CA19-9 measurement increases the effectiveness of staging laparoscopy in patients with suspected pancreatic malignancy. Dig Surg 2005; 22:80-85.

Coppola D, Lu L, Fruehauf JP et al. Analysis of p53, p21WAF1, and TGF-beta1 in human ductal adenocarcinoma of the pancreas: TGF-beta1 protein expression predicts longer survival. Am J Clin Pathol 1998; 110:16-23.

Crozier JE, McKee RF, McArdle CS et al. Preoperative but not postoperative systemic inflammatory response correlates with survival in colorectal cancer. Br J Surg 2007; 94:1028-1032.

Crumley ABC, McMillan DC, McKernan M et al. An elevated C-reactive protein concentration, prior to surgery, predicts poor cancer-specific survival in patients undergoing resection for gastro-oesophageal cancer. Br J Cancer 2006; 94:1568-1571.

Dallas NA, Fan F, Gray MJ et al. Functional significance of vascular endothelial growth factor receptors on gastrointestinal cancer cells. Cancer Metastasis Rev 2007; 26:433-441.

Dang CX, Han Y, Qin ZY et al. Clinical significance of expression of p21 and p53 proteins and proliferating cell nuclear antigen in pancreatic cancer. Hepatobiliary Pancreat Dis Int 2002; 1:302-305.

Delmotte P, Martin B, Paesmans M et al. VEGF and survival of patients with lung cancer: a systematic literature review and meta-analysis. Rev Mal Respir 2002; 19:577-584.

Dergham ST, Dugan MC, Kucway R et al. Prevalence and clinical significance of combined K-ras mutation and p53 aberration in pancreatic adenocarcinoma. Int J Pancreatol 1997; 21:127-143.

Dergham ST, Dugan MC, Joshi US et al. The clinical significance of p21(WAF1/CIP-1) and p53 expression in pancreatic adenocarcinoma. Cancer 1997; 80:372-381.

Dergham ST, Dugan MC, Sarkar FH et al. Molecular alterations associated with improved survival in pancreatic cancer patients treated with radiation or chemotherapy. J Hepatobiliary Pancreat Surg 1998; 5:269-272.

Dersimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7:177-188.

Des Guetz G, Uzzan B, Nicolas P et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer 2006; 94:1823-1832.

Dewitt J, Devereaux BM, Lehman GA et al. Comparison of endoscopic ultrasound and computed tomography for the preoperative evaluation of pancreatic cancer: a systematic review. Clin Gastroenterol Hepatol 2006; 4:717-725.

Diener MK, Heukaufer C, Schwazer G et al. Pancreaticoduodenectomy (classic Whipple) versus pylorus-preserving pancreaticoduodenectomy (pp Whipple) for surgical treatment of periampullary and pancreatic carcinoma. Cochrane Database Syst Rev. 2008; 16:CD006053.

DiGiuseppe JA, Hruban RH, Goodman SN et al. Overexpression of p53 protein in adenocarcinoma of the pancreas. Am J Clin Pathol 1994; 101:684-688.

Dong M, Nio Y, Sato Y et al. Comparative study of p53 expression in primary invasive ductal carcinoma of the pancreas between Chinese and Japanese. Pancreas 1998; 17:229-237.

Dong M, Nio Y, Guo KJ et al. Epidermal growth factor and its receptor as prognostic indicators in Chinese patients with pancreatic cancer. Anticancer Res 1998; 18:4613-4619.

Dong M, Nio Y, Tamura K et al. Ki-ras point mutation and p53 expression in human pancreatic cancer: a comparative study among Chinese, Japanese, and Western patients. Cancer Epidemiol Biomarkers Prev 2000; 9:279-284.

Dong M, Nio Y, Yamasawa K et al. p53 alteration is not an independent prognostic indicator, but affects the efficacy of adjuvant chemotherapy in human pancreatic cancer. J Surg Oncol 2003; 82:111-120.

Dong M, Ma G, Tu W et al. Clinicopathological significance of p53 and mdm2 protein expression in human pancreatic cancer. World J Gastroenterol 2005; 11:2162-2165.

Dong M, Zhou JP, Zhang H et al. Clinicopathological significance of Bcl-2 and Bax protein expression in human pancreatic cancer. World J Gastroenterol 2005; 11:2744-2747.

Doran HE, Bosonnet L, Connor S et al. Laparoscopy and laparoscopic ultrasound in the evaluation of pancreatic and periampullary tumours. Dig Surg 2004; 21:305-313.

Doucas H, Sutton CD, Zimmerman A et al. Assessment of pancreatic malignancy with laparoscopy and intraoperative ultrasound. Surg Endosc 2007; 21:1147-52.

Duell EJ, Holly EA, Bracci PM et al. A population-based case-control study of polymorphisms in carcinogen-metabolizing genes, smoking and pancreatic adenocarcinoma risk. J Natl Cancer Inst 2002; 94:297-306.

Duraker N, Hot S, Polat Y et al. CEA, CA19-9 and CA125 in the differential diagnosis of benign and malignant pancreatic disease with or without jaundice. J Surg Oncol 2007; **95:**142-147.

Dutta Roy S, Kupp DV, Slavin JP et al. Inequity of upper gastrointestinal cancer distribution and survival with socioeconomic deprivation: a population-based study. Surgery 2005; 138:859-868.
Ellis LM, Takahashi Y, Fenoglio CJ et al. Vessel counts and vascular endothelial growth factor expression in pancreatic adenocarcinoma. Eur J Cancer 1998; 34:337-340.

Ellsmere J, Moltele K, Sahari D et al. Does multidetector-row CT eliminate the role of diagnostic laparoscopy in assessing the resectability of pancreatic head adenocarcinoma? Surg Endosc 2005; 19:369-373.

Eshuis WJ, van der Gaag NA, Rauws EA et al. Therapeutic delay and survival after surgery for cancer of the pancreatic head with or without preoperative biliary drainage. Ann Surg 2010; 252:840-849.

Esposito I, Kleeff J, Bergmann F et al. Most pancreatic cancer resections are R1 resections. Ann Surg Oncol 2008; **15**:1651-1660.

Evans JD, Cornford PA, Dodson A et al. Detailed tissue expression of bcl-2, bax, bak and bcl-x in the normal human pancreas and in chronic pancreatitis, ampullary and pancreatic ductal adenocarcinomas. Pancreatology 2001; 1:254-262.

Farrell JJ, Elsaleh H, Garcia M et al. Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. Gastroenterology 2009; 136:187-195.

Ferrone CR, Finkelstein DM, Thayer SP et al. Perioperative CA19-9 levels can predict stage and survival in patients with pancreatic ductal adenocarcinoma. J Clin Oncol 2006; **24:**2897-2902.

Fitzsimmons D, Osmond C, George S et al. Trends in stomach and pancreatic cancer incidence and mortality in England and Wales, 1951-2000. Br J Surg 2007; 94:1162-1171.

Fogar P, Sperti C, Basso D et al. Decreased total lymphocyte counts in pancreatic cancer: an index of adverse outcome. Pancreas 2006; 32:22-28.

Friess H, Lu Z, Graber HU et al. bax, but not bcl-2, influences the prognosis of human pancreatic cancer. Gut 1998; 43:414-421.

Fujimoto K, Hosotani R, Wada M et al. Expression of two angiogenic factors, vascular endothelial growth factor and platelet-derived endothelial cell growth factor in human pancreatic cancer, and its relationship to angiogenesis. Eur J Cancer 1998; 34:1439-1447.

Fujioka S, Yoshida K, Yanagisawa S et al. Angiogenesis in pancreatic carcinoma: thymidine phosphorylase expression in stromal cells and intratumoral microvessel density as independent predictors of overall and relapse-free survival. Cancer 2001; 92:1788-1797.

Fukunaga A, Miyamoto M, Yasushi C et al. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. Pancreas 2004; 28:26-31.

Furukawa F, Nishikawa A, Lee IS et al. A cyclooxygenase-2 inhibitor, nimesulide, inhibits post-initiation phase of N-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters. Int J Cancer 2003; 104:269-273.

Gansauge F, Gansauge S, Schmidt E et al. Prognostic significance of molecular alterations in human pancreatic carcinoma - an immunohistological study. Langenbecks Arch Surg 1998; 383:152-155.

Gapstur SM, Jacobs EJ, Deka A et al. Association of alcohol intake with pancreatic cancer mortality in never smokers. Arch Intern Med 2011; 171:444-451.

Gansauge F, Gansauge S, Link KH et al. p53 in relation to therapeutic outcome of locoregional chemotherapy in pancreatic cancer. Ann N Y Acad Sci 1999; 880:281-287.

Garcea G, Dennison AR, Pattenden CJ et al. Survival following curative resection for pancreatic ductal adenocarcinoma: A systematic review of the literature. JOP 2008; 9:99-132

Gastrointestinal Tumour Study Group. Further evidence of effective adjuvant combined radiation and chemotherapy following curative resection of pancreatic cancer. Cancer 1987; 59:2006-2010.

Gazzaniga GM, Papadia FS, Dezzana M et al. Role of p53 mutations on survival after pancreatoduodenectomy for ductal adenocarcinoma of the pancreatic head. Hepatogastroenterology 2001; 48:1743-1745.

Gerdes B, Ramaswamy A, Ziegler A et al. p16INK4a is a prognostic marker in resected ductal pancreatic cancer: an analysis of p16INK4a, p53, MDM2, an Rb. Ann Surg 2002; 235:51-59.

Giardello FM, Offerhaus GJ, Lee DH et al. Increased risk of thyroid and pancreatic carcinoma in familial adenomatous polyposis. Gut 1993; 34:1349-1346.

Giardello FM, Brensinger JD, Tersmette AC et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000; 119:1447-1453.

Giger U, BüchlerM, Farhadi J et al. Preoperative immunonutrition suppresses perioperative inflammatory response in patients with major abdominal surgery - a randomized controlled pilot study. Ann Surg Oncol 2007; 14:2798-2806.

Glen P, Jamieson NB, McMillan DC et al. Evaluation of an inflammation-based prognostic score in patients with inoperable pancreatic cancer. Pancreatology 2006; 6:450-453.

Go VLW, Gukovskaya A, Pandol SJ. Alcohol and pancreatic cancer. Alcohol 2005; 35:205-211.

Goldstein AM, Fraser MC, Struewing JP et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with $p16^{INK4}$ mutations. N Engl J Med 1995; 333:970-974.

Haas SL, Jesnowski R, Steiner M et al. Expression of tissue factor in pancreatic adenocarcinoma is associated with activation of coagulation. World J Gastroenterol 2006; 12: 4843-9.

Hamacher R, Schmid RM, Saur D et al. Apoptotic pathways in pancreatic ductal adenocarcinoma. Mol Cancer 2008; 7:64.

Han S, Jang J, Kim S et al. Analysis of long-term survivors after surgical resection for pancreatic cancer. Pancreas 2006; **32:**271-275.

Hashimoto K, Ikeda Y, Korenaga D et al. The impact of preoperative serum C-reactive protein on the prognosis of patients with hepatocellular carcinoma. Cancer 2005; 103:1856-1864.

Hashimoto K, Nio Y, Koike M et al. Expression of retinoblastoma and p53 pathway-related proteins in resectable invasive ductal carcinoma of the pancreas: potential cooperative effects on clinical outcome. Anticancer Res 2005; 25:1361-1368.

Hayden JA, Côté P, Bombardier C. Evaluation of the quality of prognosis studies in systematic reviews. Ann Intern Med 2006; 144:427-437.

Hermanova M, Karasek P, Nenuti R et al. Clinicopathological correlations of cyclooxygenase-2, MDM2, and p53 expressions in surgically resectable pancreatic invasive ductal adenocarcinoma. Pancreas 2009; 38:565-571.

Higgins J, Thompson J, Deeks J et al. Measuring inconsistency in meta-analyses. BMJ 2003; 327:557-560.

Holländer N, Shumacher M. On the problem of using 'optimal' cutpoints in the assessment of quantitative prognostic factors. Onkologie 2001; 24:194-199.

Holländer N, Sauerbrei W, Schumacher M. Confidence intervals for the effect of a prognostic factor after selection of an 'optimal' cutpoint. Stat Med 2004; 23:1701-1713.

Howes N, Lerch MM, Greenhalf W et al. Clinical and genetic characteristics of hereditary pancreatitis in Europe. Clin Gastroenterol Hepatol 2004; 2:252-256.

Howes N, Greenhalf W, Stocken DD et al. Cationic trypsinogen mutations and pancreatitis. Clin Lab Med 2005; 25:39-59.

Hruban RH, Petersen GM, Ha PK et al. Genetics of pancreatic cancer. From genes to families. Surg Oncol Clin N Am 1998; 7:1-23.

Hu YX, Watanabe H, Ohtsubo K et al. Frequent loss of p16 expression and its correlation with clinicopathological parameters in pancreatic carcinoma. Clin Cancer Res 1997; 3:1473-1477.

Huxley R, Ansary-Moghaddam A, Berrington de Gonzalez A et al. Type II diabetes and pancreatic cancer: a meta-analysis of 36 studies. Br J Cancer 2005; 92:2076-83.

Hu YX, Watanabe H, Ohtsubo K et al. Bcl-2 expression related to altered p53 protein and its impact on the progression of human pancreatic carcinoma. Br J Cancer 1999; 80:1075-1079.

Hua Z, Zhang YC, Hu XM et al. Loss of DPC4 expression and its correlation with clinicopathological parameters in pancreatic carcinoma. World J Gastroenterol 2003; 9:2764-2767.

Ikeda N, Adachi M, Taki T et al. Prognostic significance of angiogenesis in human pancreatic cancer. Br J Cancer 1999; 79:1553-1563

Ikeda N, Nakajima Y, Sho M et al. The association of K-ras gene mutation and vascular endothelial growth factor gene expression in pancreatic carcinoma. Cancer 2001; 92:488-499.
Inoue K, Nakane Y, Iiyama H et al. The superiority of ratio-based lymph node staging in gastric carcinoma. Ann Surg Oncol 2002; 9:27-34.

International Agency for Research on Cancer. GLOBOCAN 2002. Cancer incidence, mortality and Prevalence Worldwide (2002 estimates). http://www-dep.iarc.fr/

Itakura J, Ishiwata T, Friess H et al. Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. Clin Cancer Res 1997; 3:1309-1316.

Itzjowitz S, Kim Y. New carbohydrate tumour markers. Gastroenterology 1986; 90:491-494.

Jacobs EJ, Connell CJ, Rodriguez C et al. Aspirin use and pancreatic cancer mortality in a large United States cohort. J Natl Cancer Inst 2004; 96:524-528.

Jadad AR, Moore RA, Carroll DD, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomised clinical trials: is blinding necessary? Control Clin Trials 1996; 17:1-12.

Jagannath P, Dhir V, Shrikhande S et al. Effect of preoperative biliary stenting on immediate outcome after pancreaticoduodenectomy. Br J Surg 2005; 92:356-361.

Jamieson NB, Glen P, McMillan DC et al. Systemic inflammatory response predicts outcome in patients undergoing resection for ductal adenocarcinoma head of pancreas. Br J Cancer 2005; 17:21-23.

Japan Pancreas Society. Classification of Pancreatic Carcinoma (2nd ed). Kanehara: Tokyo, 2003.

Jarufe NP, Coldham C, Mayer AD et al. Favourable prognostic factors in a large UK experience of adenocarcinoma of the head of the pancreas and periampullary region. Dig Surg 2004; **21**:202-209.

Jelski W, Chrostek L, Szmitkowski M. The activity of class I, II, III and IV of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in pancreatic cancer. Pancreas 2007; 35:142-146.

Jeong J, Park YN, Park JS et al. Clinical significance of p16 protein expression loss and aberrant p53 protein expression in pancreatic cancer. Yonsei Med J 2005; 46:519-525.

Jethwa P, Breuning E, Bhati C et al. The microbiological impact of pre-operative biliary drainage on patients undergoing hepato-biliary-pancreatic (HPB) surgery. Aliment Pharmacol Ther 2007; 25:1175-1180.

Ji J, Hemminki K. Socioeconomic and occupational risk factors for pancreatic cancer: a cohort study in Sweden. J Occup Environ Med 2006; 48:283-288.

Jinfeng M, Kimura W, Sakurai F et al. Prognostic role of angiogenesis and its correlations with thymidine phosphorylase and p53 expression in ductal adenocarcinoma of the pancreas. Hepatogastroenterology 2007; 54:1635-1640.

Kalser MH, Ellenberg SS. Pancreatic cancer: adjuvant combined radiation and chemotherapy following curative resection. Arch Surg 1985; 120:899-903.

Karachristos A, Scarmeas N, Hoffman JP. CA19-9 levels predict results of staging laparoscopy in pancreatic cancer. J Gastrointest Surg 2005; 9:1286-1292.

Karademir S, Sökmen S, Terzi C et al. Tumor angiogenesis as a prognostic predictor in pancreatic cancer. J Hepatobiliary Pancreat Surg 2000; 7:489-495.

Kawesha A, Ghaneh P, Andrén-Sandberg A et al. K-ras oncogene subtype mutations are associated with survival but not expression of p53, p16(INK4A), p21(WAF-1), cyclin D1, erbB-2 and erbB-3 in resected pancreatic ductal adenocarcinoma. Int J Cancer 2000; 89:469-474.

Kau SY, Shyr YM, Su CH et al. Diagnostic and prognostic values of CA19-9 and CEA in periampullary cancers. J Am Coll Surg 1999; **188:**415-420.

Kayahara M, Nagakawa T, Ueno K et al. An evaluation of radical resection for pancreatic cancer based on the mode of recurrence as determined by autopsy and diagnostic imaging. Cancer 1993; **72**:2118-2223.

Ker CG, Chen JS, Lee KT et al. Assessment of serum and bile level of CA19-9 and CA125 in cholangitis and bile duct carcinoma. J Gastroenterol Hepatol 1991; **6:**505-508.

Kernan GJ, Ji BT, Dosemeci M et al. Occupational risk factors for pancreatic cancer: a case control study based on death certificates from 24 US states. Am J Ind Med 1999; 36:260-270.

Khorana AA, Fine RL. Pancreatic cancer and thromboembolic disease. Lancet Oncol 2004; 5: 655-663.

Khorana AA, Hu YC, Ryan CK et al. Vascular endothelial growth factor and DPC4 predict adjuvant therapy outcomes in resected pancreatic cancer. J Gastrointest Surg 2005; 9:903-911.

Khorana AA, Ahrendt SA, Ryan CK et al. Tissue Factor expression, angiogenesis and thrombosis in pancreatic cancer. Clin Cancer Res 2007; 13:2870-2875.

Khurana V, Sheth A, Caldito G et al. Statins reduce the risk of pancreatic cancer in humans: a case-control study of half a million veterans. Pancreas 2007; 34:260-265.

Kim HJ, Kim MH, Myung SJ et al. A new strategy for the application of CA19-9 in the differentiation of pancreato-biliary cancer: Analysis using a receiver operating characteristic curve. Am J Gastroenterol 1999; **94:**1941-1946.

Klinger MH, Jelkmann W. Role of blood platelets in infection and inflammation. J Interferon Cytokine Res 2002; 22:913-922.

Klinkenbijl JH, Jeekel J, Sahmoud T et al. Adjuvant radiotherapy and 5-fluorouracil after curative resection of cancer of the pancreas and periampullary region: Phase III trial of the EORTC Gastrointestinal Tract Cancer Cooperative Group. Ann Surg 1999; 230:776-784.

Kloppel G, Solcia E, Longnecker DS et al. Histological typing of tumours of the exocrine pancreas, 2nd ed. WHO international histological classification of tumours. Berlin: Springer-Verlag, 1996.

Knoll MR, Rudnitzki D, Sturm J et al. Correlation of postoperative survival and angiogenic growth factors in pancreatic carcinoma. Hepatogastroenterology 2001; 48:1162-1165.

Kunnumakarra AB, Guha S, Krishnan S et al. Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products. Cancer Res 2007; 67:3853-3861.

Kurahara H, Takao S, Maemura K et al. Impact of vascular endothelial growth factor-C and -D expression in human pancreatic cancer: its relationship to lymph node metastasis. Clin Cancer Res 2004; 10:8413-8420.

Kusama T, Mukai M, Iwasaki T et al. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors reduce human pancreatic cancer cell invasion and metastasis. Gastroenterology 2002; 122:308-317.

Kuwahara K, Sasaki T, Kuwada Y et al. Expressions of angiogenic factors in pancreatic ductal carcinoma: a correlative study with clinicopathologic parameters and patient survival. Pancreas 2003; 26:344-349.

Kyzas PA, Cunha IW, Ioannidis JP. Prognostic significance of vascular endothelial growth factor immunohistochemical expression in head and neck squamous cell carcinoma: a meta-analysis. Clin Cancer Res 2005; 11:1434-1440.

Kyzas PA, Loizou KT, Ioannidis JP. Selective reporting biases in cancer prognostic factor studies. J Natl Cancer Inst 2005; 97:1043-1055.

Lee EU, Cibull ML, O'Daniel-Pierce E et al. Expression of p53 protein in pancreatic adenocarcinoma. Relationship to cigaret smoking. Int J Pancreatol 1995; 17:237-42.

Lev-Ari S, Starr A, Vexler A et al. Inhibition of pancreatic and lung adenocarcinoma cell survival by curcumin is associated with increased apoptosis, down-regulation of COX-2 and EGFR and inhibition Erk 1/2 activity. Anticancer Res 2006; 26:4423-4430.

Li D, Day RS, Bondy ML et al. Dietary mutagen exposure and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Prev 2007; 16:655-661.

Li Y, Bhuiyan M, Vaitkevicius VK et al. Structural alteration of p53 protein correlated to survival in patients with pancreatic adenocarcinoma. Pancreas 1999; 18:104-110.

Lim YJ, Lee JK, Park CK et al. Prognostic value of VEGF in human pancreatic ductal adenocarcinoma. Korean J Intern Med 2004; 19:10-14.

Lin Y, Tamakoshi A, Kawamura T et al. A prospective cohort study of cigarette smoking and pancreatic cancer in Japan. Cancer Causes Control 2002; 13:249-254.

Lin Y, Tamakoshi A, Hayakawa T et al. Nutritional factors and risk of pancreatic cancer: a population-based case-control study based on direct interview in Japan. J Gastroenterol 2005; 40:297-301.

Linder S, Parrado C, Falkmer UG. Prognostic significance of Ki-67 antigen and p53 protein expression in pancreatic duct carcinoma: a study of the monoclonal antibodies MIB-1 and DO-7 in formalin-fixed paraffin-embedded tumour material. Br J Cancer 1997; 76:54-59.

Linder S, Blåsjö M, von Rosen A et al. Pattern of distribution and prognostic value of angiogenesis in pancreatic duct carcinoma: a semiquantitative immunohistochemical study of 45 patients. Pancreas 2001; 22:240-247.

Lowenfels AB, Maisonneuve P, DiMagno EP et al. Hereditary pancreatitis and the risk of pancreatic cancer. J Natl Cancer Inst 1997; 89:442-446.

Lundin J, Roberts PJ, Kuusela P et al. The prognostic value of preoperative serum levels of CA19-9 and CEA in patients with pancreatic cancer. Br J Cancer 1994; 69:515-519.

Lundin J, Nordling S, von Boguslawsky K et al. Prognostic value of immunohistochemical expression of p53 in patients with pancreatic cancer. Oncology 1996; 53:104-111.

Lyman GH, Kuderer NM. The strengths and limitations of meta-analyses based on aggregate data. BMC Med Res Methodol 2005; 5:14.

Magistrelli P, Coppola R, Tonini G et al. Apoptotic index or a combination of Bax/Bcl-2 expression correlate with survival after resection of pancreatic adenocarcinoma. J Cell Biochem 2006; 97:98-108.

Maisonneuve P, Lowenfels AB. Chronic pancreatitis and pancreatic cancer. Dig Dis 2002; 20:32-37.

Mäkinen K, Hakala T, Lipponen P et al. Clinical contribution of bcl-2, p53 and Ki-67 proteins in pancreatic ductal adenocarcinoma. Anticancer Res 1998; 18:615-658.

Malats N, Bustos A, Nascimento CM et al. P53 as a prognostic marker for bladder cancer: a meta-analysis and review. Lancet Oncol 2005; 6:678-686.

Manuyakorn A, Paulus R, Farrell J et al. Cellular histone modification patterns predict prognosis and treatment response in resectable pancreatic adenocarcinoma: results from RTOG 9704. *J Clin Oncol* 2010; **28:**1358-65.

Martin B, Paesmans M, Berghmans T et al. Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer 2003; 89:55-64.

McFaul CD, Greenhalf W, Earl J et al. Anticipation in familial pancreatic cancer. Gut 2006; 55:252-258.

McShane LM, Altman DG, Sauerbrei W et al. Reporting recommendations for tumor marker prognostic studies (REMARK) Exp Oncol 2006; 28:99-105.

Minnard EA, Conlon KC, Hoos A et al. Laparoscopic ultrasound enhances standard laparoscopy in the staging of pancreatic cancer. Ann Surg 1998; 228:182-187.

Moher D, Shulz K, Altman D. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. Lancet 2001; 357:1191-1194.

Montgomery RC, Hoffman JP, Riley LB et al. Prediction of recurrence and survival by post-resection CA19-9 values in patients with adenocarcinoma of the pancreas. Ann Surg Oncol 1997; **4:**551-556.

Moon HJ, An JY, Heo JS et al. Predicting survival after surgical resection for pancreatic ductal adenocarcinoma. Pancreas 2006; **32:**37-43.

Nagakawa T, Nagamori M, Futakami F et al. Results of extensive surgery for pancreatic carcinoma. Cancer 1996; **77**:640-645.

Naka T, Kobayashi M, Ashida K et al. Aberrant p16INK4 expression related to clinical stage and prognosis in patients with pancreatic cancer. Int J Oncol 1998; 12:1111-1116.

Nakagohri T, Kinoshita T, Konishi M et al. Survival benefits of portal vein resection for pancreatic cancer. Am J Surg 2003; 186:149-153.

Nash AD, Baca M, Wright C et al. The biology of vascular endothelial growth factor-B (VEGF-B). Pulm Pharmacol Ther 2006; 19:61-69.

Neoptolemos JP, Russell RC, Bramhall S et al. Low mortality following resection for pancreatic and periampullary tumours in 1026 patients: UK survey of specialist pancreatic units. UK Pancreatic Cancer Group. Br J Surg 1997; 84:1370-1376.

Neoptolemos JP, Stocken DD, Dunn JA et al. European Study Group for Pancreatic Cancer. Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. Ann Surg 2001; **234**:758-768.

Neoptolemos JP, Stocken DD, Friess H et al. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. N Engl J Med 2004; 350:1200-1210.

Neoptolemos JP, Stocken DD, Ghaneh et al. European Study Group for Pancreatic Cancer. Adjuvant chemotherapy with flurouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. JAMA 2010; 304: 1073-1081.

Niedergethmann M, Hildenbrand R, Wolf G et al. Angiogenesis and cathepsin expression are prognostic factors in pancreatic adenocarcinoma after curative resection. Int J Pancreatol 2000; 28:31-39.

Niedergethmann M, Hildenbrand R, Wostbrock B et al. High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas. Pancreas 2002; 25:122-9.

Nieveen van Dijkum EJ, Romijn MG et al. Laparoscopic staging and subsequent palliation in patients with peripancreatic carcinoma. Ann Surg 2003; 237:66-73.

Nio Y, Dong M, Uegaki K et al. p53 expression affects the efficacy of adjuvant chemotherapy after resection of invasive ductal carcinoma of the pancreas. Anticancer Res 1998; 18:3773-3779.

Nio Y, Dong M, Uegaki K et al. Comparative significance of p53 and WAF/1-p21 expression on the efficacy of adjuvant chemotherapy for resectable invasive ductal carcinoma of the pancreas. Pancreas 1999; 18:117-126.

Nio Y, Iguchi C, Yamasawa K et al. Apoptosis and expression of Bcl-2 and Bax proteins in invasive ductal carcinoma of the pancreas. Pancreas 2001; 22:230-239.

Nio Y, Dong M, Iguchi C et al. Expression of Bcl-2 and p53 protein in resectable invasive ductal carcinoma of the pancreas: effects on clinical outcome and efficacy of adjuvant chemotherapy. J Surg Oncol 2001; 76:188-96.

Nitori N, Ino Y, Nakanishi T et al. Prognostic significance of tissue factor in pancreatic ductal adenocarcinoma. Clin Cancer Res 2005; 11:2531-2539.

Nozoe T, Saeki H, Sugimachi K. Significance of preoperative elevation of serum C-reactive protein as an indicator of prognosis in esophageal carcinoma. Am J Surg 2001; 182:197-201.

Oettle H, Post S, Neuhaus P et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 2007; 297:267-277.

Office for National Statistics. Mortality Statistics: Cause. Review of the Registrar General on Deaths by Cause, Sex and Age in England and Wales, 2003. Series DH2 no.30. Office for National Statistics: London, 2004.

Office for National Statistics. Health Statistics Quarterly, Autumn 2007, No. 35. http://www.statistics .gov/

Ohshio G, Suwa H, Imamura T et al. An immunohistochemical study of bcl-2 and p53 protein expression in pancreatic carcinomas. Scand J Gastroenterol 1998; 33:535-539.

Ohshio G, Suwa H, Imamura M. Clinical implication of anti-p53 antibodies and p53-protein in pancreatic disease. Int J Gastrointest Cancer 2002; 31:129-135.

Ohtsubo K, Watanabe H, Yamaguchi Y et al. Abnormalities of tumor suppressor gene p16 in pancreatic carcinoma: immunohistochemical and genetic findings compared with clinicopathological parameters. J Gastroenterol 2003; 38:663-671.

Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival end-points. Stat Med 1998; 17:2815-2834.

Pawlik TM, Gleisner AL, Cameron JL et al. Prognostic relevance of lymph node ratio following pancreaticoduodenectomy for pancreatic cancer. Surgery 2007; 14:610-618.

Peltomaki P, Vasen HF. Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. Gastroenterology 1997; 113:1146-1158.

Phelan CM, Lancaster JM, Tonin P et al. Mutation analysis of the BRCA2 gene in 49 site-specific breast cancer families. Nat Genet 1996; 8:392-398.

Piantadosi S. Clinical trials: A methodological perspective, 2nd Edition, Wiley & Sons; 2005.

Prokopczyk B, Hoffman D, Bologna M et al. Identification of tobacco-derived compounds in human pancreatic juice. Chemical Res Toxicol 2002; 15:677-685.

Raut CP, Tseng JF, Sun CC et al. Impact of resection status on pattern of failure and survival after pancreaticoduodenectomy for pancreatic adenocarcinoma. Ann Surg 2007; **246**:52-60.

Riall TS, Cameron JL, Lillemoe KD et al. Resected periampullary adenocarcinoma: 5-year survivors and their 6- to 10-year follow-up. Surgery 2006; 140:764-772.

Ries LAG, Eisner MP, Kosary CL et al. SEER Cancer Statistics Review 1973-1999. Bethesda: National Cancer Institute, 2002.

Romano F, Uggeri F, Crippa S et al. Immunodeficiency in different histotypes of radically operable gastrointestinal cancers. J Exp Clin Cancer Res 2004; 23:195-200.

Safi F, Schlosser W, Falkenreck S et al. Prognostic value of CA19-9 serum course in pancreatic cancer. Hepatogastroenterology 1998; **45:**253-259.

Salazar-Onfray F, Lopez MN, Mendoza-Naranjo A. Paradoxical effects of cytokines in tumor immune surveillance and tumor escape. Cytokine & Growth Factor Reviews 2007; 18:171-182.

Sarela AI, Verbeke CS, Ramsdale J et al. Expression of survivin, a novel inhibitor of apoptosis and cell cycle regulatory protein, in pancreatic adenocarcinoma. Br J Cancer 2002; 86:886-892.

Sato Y, Nio Y, Song MM et al. p53 protein expression as prognostic factor in human pancreatic cancer. Anticancer Res 1997; 17:2779-2788.

Schernhammer ES, Kang JH, Chan JH et al. A prospective study of aspirin use and the risk of pancreatic cancer in women. J Natl Cancer Inst 2004; 96:22-28.

Schlieman MG, Ho HS, Bold RJ. Utility of tumour markers in determining resectability of pancreatic cancer. Arch Surg 2003; 138:951-956.

Schmidt CM, Powell ES, Yiannoutsos CT et al. Pancreaticoduodenectomy: a 20-year experience in 516 patients. Arch Surg 2004; 139:718-725.

Schulze J, Richter E, Binder U et al. Biliary excretion of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in the rat. Carcinogenesis 1992; 13:1961-1965.

Schutte M, Hruban RH, Geradts J. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. Cancer Res 1997; 57:3126-3130.

Schwarz RE, Keny H. Preoperative platelet count predicts survival after resection of periampullary adenocarcinoma. Hepatogastroenterology 2001; 48:1493-1498.

Seo Y, Baba H, Fukuda T et al. High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. Cancer 2000; 88:2239-2245.

Sessa F, Bonato M, Bisoni D et al. Ki-ras and p53 gene mutations in pancreatic ductal carcinoma: a relationship with tumor phenotype and survival. Eur J Histochem 1998; 42:67-76.

Sewnath ME, Karsten TM, Prins MH et al. A meta-analysis on the efficacy of preoperative biliary drainage for tumors causing obstructive jaundice. Ann Surg 2002; 236:17-27.

Shah SM, Carey IM, Owen CG et al. Does beta-adrenoceptor blocker therapy improve cancer survival? Findings from a population-based retrospective cohort study. Br J Clin Pharmacol 2011; 72:157-161.

Sierko E, Wojtukiewicz MZ. Platelets and angiogenesis in malignancy. Semin Thromb Hemost 2004; 30:95-108.

Silverman DT, Dunn JA, Hoover RN et al. Cigarette smoking and pancreas cancer: a case-control study based on direct interviews. J Natl Cancer Inst 1994; 86:1510-1516.

Silverman DT, Swanson CA, Gridley G et al. Dietary and nutritional factors and pancreatic cancer: a case-control study based on direct interviews. J Natl Cancer Inst 1998; 90:1710-1719.

Silverman DT, Hoover RN, Brown LM et al. Why do black Americans have a higher risk of pancreatic cancer than white Americans? Epidemiology 2003; 14:45-54.

Sinicrope FA, Evans DB, Leach SD et al. bcl-2 and p53 expression in resectable pancreatic adenocarcinomas: association with clinical outcome. Clin Cancer Res 1996; 2:2015-2022.

Slidell MB, Chang DC, Cameron JL et al. Impact of total lymph node count and lymph node ratio on staging and survival after pancreatectomy for pancreatic adenocarcinoma: A large, population-based analysis. Ann Surg Oncol 2008; 15: 165-174.

Smeenk HG, Erdmann J, van Dekken H et al. Long-term survival after radical resection for pancreatic head and ampullary cancer: a potential role for the EGF-R. Dig Surg 2007; 24:38-45.

Smith RA, Ghaneh P, Sutton R et al. Prognosis of resected ampullary adenocarcinoma by preoperative serum CA19-9 levels and platelet-lymphocyte ratio. J Gastrointest Surg 2008; 12:1422-1428.

Sperti C, Pasquali C, Catalini S et al. CA19-9 as a prognostic index after resection for pancreatic cancer. J Surg Oncol 1993; **52:**137-141.

Sperti C, Pasquali C, Piccoli A et al. Recurrence after resection for ductal adenocarcinoma of the pancreas. World J Surg 1997; **21**:195-200.

Stan SD, Singh SS, Brand RE. Chemoprevention strategies for pancreatic cancer. Nat Rev Gastroenterol Hepatol 2010; 7:347-356.

Stevens RJ, Roddam AW, Beral V. Pancreatic cancer in type 1 and young onset diabetes: systematic review and meta-analysis. Br J Cancer 2007; 96:507-509.

Stipa F, Lucandri G, Limiti MR et al. Angiogenesis as a prognostic indicator in pancreatic ductal adenocarcinoma. Anticancer Res 2002; 22:445-9.

Stocken DD, Büchler MW, Dervenis C et al. Meta-analysis of randomised adjuvant therapy trials for pancreatic cancer. *Br J Cancer* 2005; 92:1372-1382.

Sultana A, Tudor-Smith C, Cunningham D et al. Systematic review, including meta-analysis, on the management of locally advanced pancreatic cancer using radiation/combined modality therapy. Br J Cancer 2007; 96:1183-1190.

Sun CY, Wang BL, Hu CQ et al. Expression of the bcl-2 gene and its significance in human pancreatic carcinoma. Hepatobiliary Pancreat Dis Int 2002; 1:306-308.

Suzuki K, Aiura K, Ueda M et al. The influence of platelets on the promotion of invasion by tumor cells and inhibition by antiplatelet agents. Pancreas 2004; 29:132-140.

Suzuki K, Aiura K, Kitagou M et al. Platelet counts closely correlate with the disease-free survival interval of pancreatic cancer patients. Hepatogastroenterology 2004a; 51:847-853.

Takada A, Amano H, Yasuda H et al. Is postoperative adjuvant chemotherapy useful for gallbladder carcinoma? A Phase III multicenter prospective randomized controlled trial in patients with resected pancreaticobiliary carcinoma. Cancer 2002; 95:1685-1695.

Takikita M, Altekruse S, Lynch CF et al. Associations between selected biomarkers and prognosis in a population-based pancreatic cancer tissue microarray. Cancer Res 2009; 69:2950-2955.

Tascilar M, Skinner HG, Rosty C et al. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. Clin Cancer Res 2001; 7:4115-4121.

Tanaka M, Chari S, Adsay V et al. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. Pancreatology 2006; 6:17-32.

Tang RF, Wang SX, Peng L et al. Expression of vascular endothelial growth factors A and C in human pancreatic cancer. World J Gastroenterol 2006; 12:280-286.

Tang RF, Itakura J, Aikawa T et al. Overexpression of lymphangiogenic growth factor VEGF-C in human pancreatic cancer. Pancreas 2001; 22:285-292.

Thomson BN, Parks RW, Redhead DN et al. Refining the role of laparoscopy and laparoscopic ultrasound in the staging of presumed pancreatic head and ampullary tumours. Br J Cancer 2006; 94:213-217.

Tierney JF, Stewart LA, Ghersi D. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007 8:16.

Toga T, Nio Y, Hashimoto K et al. The dissociated expression of protein and messenger RNA of DPC4 in human invasive ductal carcinoma of the pancreas and their implication for patient outcome. Anticancer Res 2004; 24:1173-1178.

Tomazic A, Pegan V, Ferlan-Marolt K et al. Cyclin D1 and bax influence the prognosis after pancreatoduodenectomy for periampullary adenocarcinoma. Hepatogastroenterology 2004; 51:1832-1837.

Ueda S, Ogata S, Tsuda H et al. The correlation between cytoplasmic overexpression of epidermal growth factor receptor and tumor aggressiveness: poor prognosis in patients with pancreatic ductal adenocarcinoma. Pancreas 2004; 29:1-8.

Ueda S, Hatsuse K, Tsuda H et al. Potential crosstalk between insulin-like growth factor receptor type 1 and epidermal growth factor receptor in progression and metastasis of pancreatic cancer. Mod Pathol 2006; 19:788-796.

Uegaki K, Nio Y, Inoue Y et al. Clinicopathological significance of epidermal growth factor and its receptor in human pancreatic cancer. Anticancer Res 1997; 17:3841-3847.

van der Gaag NA, Rauws EA, van Eijck CH et al. Preoperative biliary drainage for cancer of the head of the pancreas. NEJM 2010; 362: 129-137.

van Geenan RC, ten Kate FJ, de Wit LT et al. Segmental resection and wedge excision of the portal or superior mesenteric vein during pancreatoduodenectomy. Surgery 2001; 129:158-163.

Verbeke CS, Leitch D, Menon KV et al. Redefining the R1 resection in pancreatic cancer. Br J Surg 2006; **93:**1232-1237.

Virkajärvi N, Pääkkö P, Soini Y. Association between p53 overexpression, cell proliferation, tumor necrosis and extent of apoptosis in operated pancreatic adenocarcinoma. APMIS 1997; 105:765-772.

Vitone LJ, Greenhalf W, Howes N et al. Hereditary pancreatitis and secondary screening for early pancreatic cancer. Rocz Akad Med Bialymst 2005; 50:73-84.

von Bernstorff W, Voss M, Freichel S et al. Systemic and local immunosuppression in pancreatic cancer patients. Clin Cancer Res 2001; 7(Suppl):925s-932s.

von Ritter C, Eder MI, Stieber P et al. Biliary mucin secreted by cultured human gallbladder epithelial cells carries the epitope of CA19-9. Anticancer Res 1997; **17:**2931-2934.

Wang Z, Zhang Y, Banerjee S et al. Notch-1 down-regulation by curcumin is associated with the inhibition of cell growth and the induction of apoptosis in pancreatic cancer cells. Cancer 2006; 106:2503-2513.

Weiderpass E, Pukkala E. Time trends in socioeconomic differences in incidence rates of cancers of gastro-intestinal tract in Finland. BMC Gastroenterol 2006; 6:41.

Wenger FA, Kilian M, Bisevac M et al. Effects of Celebrex and Zyflo on liver metastasis and lipid peroxidation in pancreatic cancer in Syrian hamsters. Clin Exp Metastasis 2002; 19:681-687.

Westerdahl J, Andren-Sandberg A, Ihse I. Recurrence of exocrine pancreatic cancer: local or hepatic? Hepatogastroenterology 1993; **40**:384-387.

Westphal S, Kalthoff H. Apoptosis: targets in pancreatic cancer. Mol Cancer 2003; 2:6.

Whitcomb DC, Gorry M, Preston R et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 1996; 4:141-145.

Whitcomb DC, Pogue-Geile K. Pancreatitis as a risk factor for pancreatic cancer. Gastroenterol Clin North Am 2002; 31:663-678.

Wilentz RE, Iacobuzio-Donahue CA, Argani P. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. Cancer Res 2000; 60:2002-2006.

Williamson PR, Smith CT, Hutton JL, Marson AG. Aggregate data meta-analysis with time-to-event outcomes. *Stat Med* 2002; **21:**3337-51.

Winter JM, Cameron JL, Campbell KA et al. 1423 pancreaticoduodenectomies for pancreatic cancer: A single-institution experience. J Gastrointest Surg 2006; **10**:1199-1210.

Winter JM, Cameron JL, Yeo CJ et al. Biochemical markers predict morbidity and mortality after pancreaticoduodenectomy. J Am Coll Surg 2007; 204:1029-1036.

Wood HE, Gupta S, Kang JY et al. Pancreatic cancer in England and Wales 1975-200: patterns and trends in incidence, survival and mortality. Aliment Pharmacol Ther 2006; 23:1205-1214.

Woutersen RA, Appel MJ, van Garderen-Hoetmer A. Modulation of pancreatic carcinogenesis by antioxidants. Food Chem Toxicol 1999; 37:981-984.

Wynder EL, Mabuchi K, Maruchi N et al. Epidemiology of cancer of the pancreas. J Natl Cancer Inst 1973; 50:645-667.

Yamaguchi K, Chijiiwa K, Torato N et al. Ki-ras codon 12 point and P53 mutations: a molecular examination of the main tumor, liver, portal vein, peripheral arterial blood and para-aortic lymph node in pancreatic cancer. Am J Gastroenterol 2000; 95:1939-1945.

Yamaguchi K, Noshiro H, Shimizu S et al. Long-term and short-term survivors after pancreatectomy for pancreatic cancer. Int Surg 2000; 85:71-76.

Yamanaka Y, Friess H, Kobrin MS et al. Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. Anticancer Res 1993; 13:565-569.

Yamasawa K, Nio Y, Dong M et al. Clinicopathological significance of abnormalities in Gadd45 expression and its relationship to p53 in human pancreatic cancer. Clin Cancer Res 2002; 8:2563-2569.

Yamazaki Y, Morita T. Molecular and functional diversity of vascular endothelial growth factors. Mol Divers 2006; 10:515-527.

Ye W, Lagergren J, Weidemass E et al. Alcohol abuse and the risk of pancreatic cancer. Gut 2002; 51:236-239.

Yokoyama M, Yamanaka Y, Friess H et al. p53 expression in human pancreatic cancer correlates with enhanced biological aggressiveness. Anticancer Res 1994; 14:2477-2483.

Yu G, Zhu MH, Zhu Z et al. Expression of ATM protein and its relationship with p53 in pancreatic carcinoma with tissue array. Pancreas 2004; 28:421-426.

Zhang SY, Ruggeri B, Agarwal P et al. Immunohistochemical analysis of p53 expression in human pancreatic carcinomas. Arch Pathol Lab Med 1994; 118:150-154.

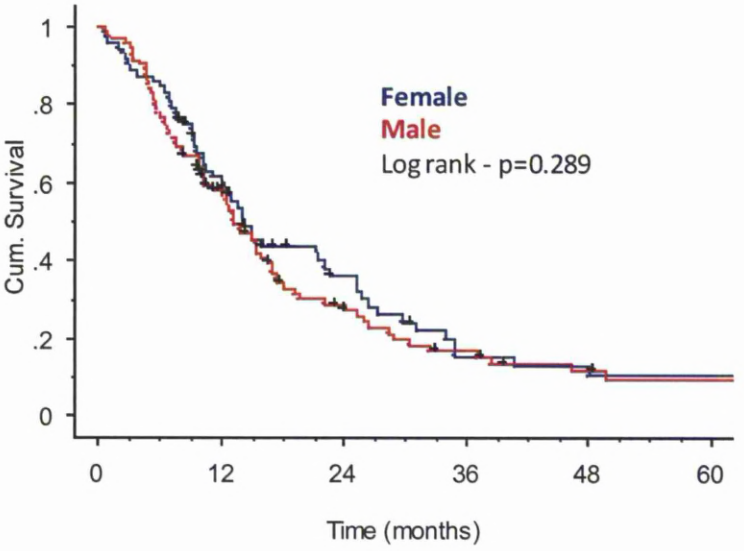
Zhang L, Yuan SZ. Expression of c-erbB-2 oncogene protein, epidermal growth factor receptor, and TGF-beta1 in human pancreatic ductal adenocarcinoma. Hepatobiliary Pancreat Dis Int 2002; 1:620-623.

Zhang B, Zhao WH, Zhou WY et al. Expression of vascular endothelial growth factors-C and -D correlate with evidence of lymphangiogenesis and angiogenesis in pancreatic adenocarcinoma. Cancer Detect Prev 2007; 31:436-442.

Zhang D, Ma Q, Wang Z et al. Beta2-adrenoceptor blockage induces G1/S phase arrest and apoptosis in pancreatic cancer cells via Ras/Akt/NFkappaB pathway.

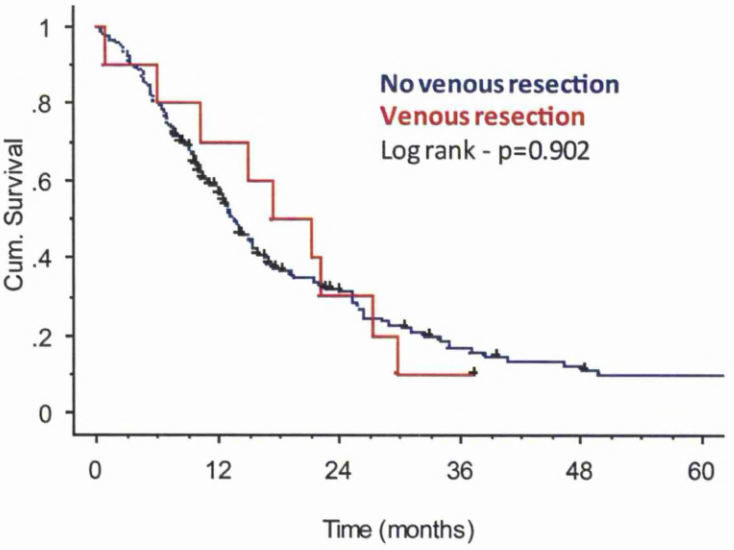
APPENDIX A - *Supplementary figures / tables*

fig.2A - Kaplan-Meier cumulative survival curves for resected pancreatic ductal adenocarcinoma patients according to gender.



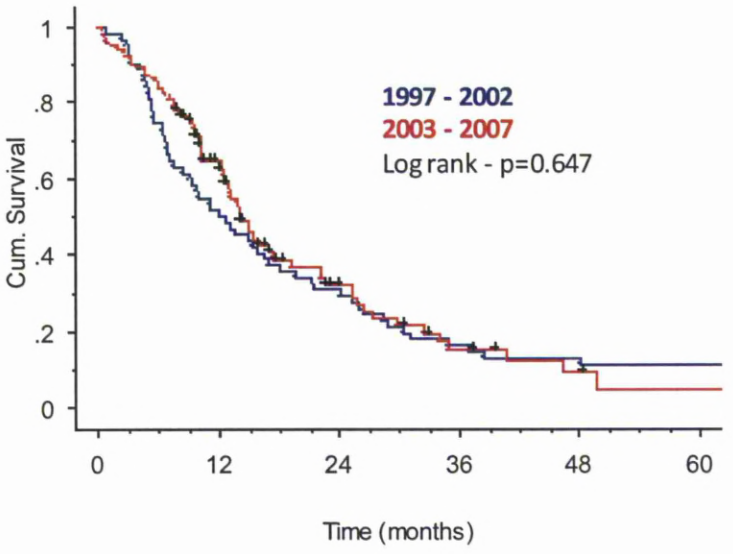
No. at risk						
Male	94	47	20	10	6	4
Female	72	41	18	7	5	4

fig.4A - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to venous resection.



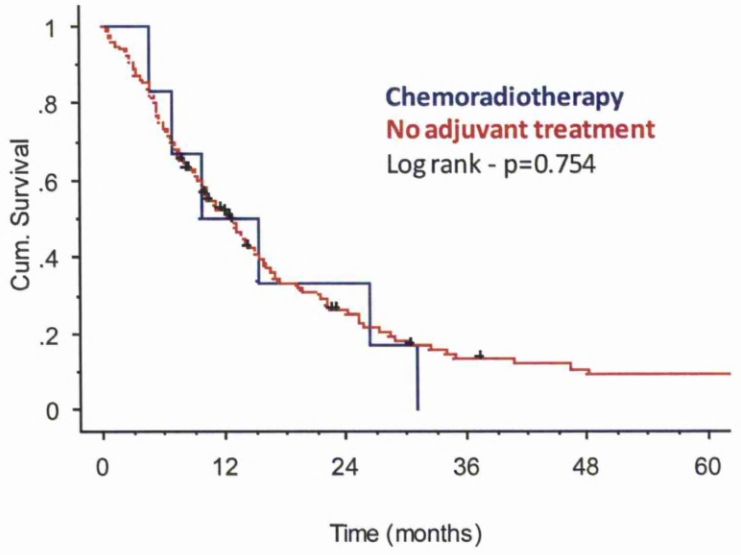
No. at risk						
None	156	81	35	16	11	8
Venous res.	10	7	3	1	0	0

fig.5A - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to study period.



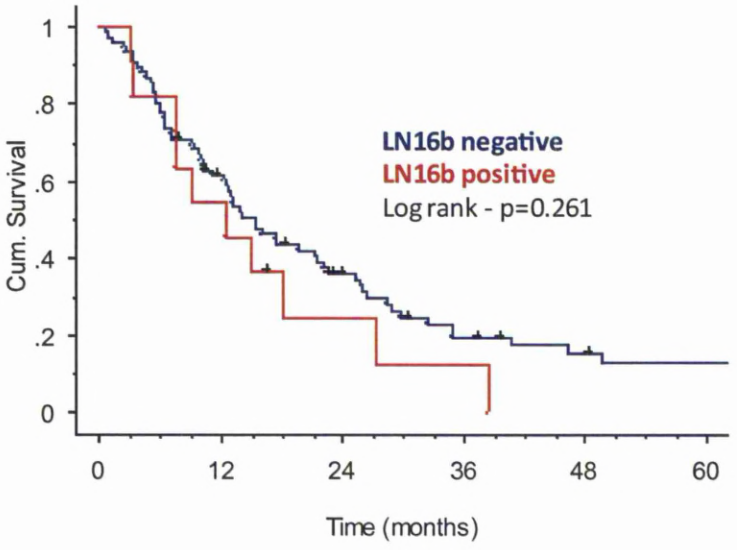
No. at risk						
1997 - 2002	62	32	19	10	8	7
2003 - 2007	104	56	19	7	3	1

fig.7A - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to adjuvant chemoradiotherapy (CRT) vs. no adjuvant therapy.



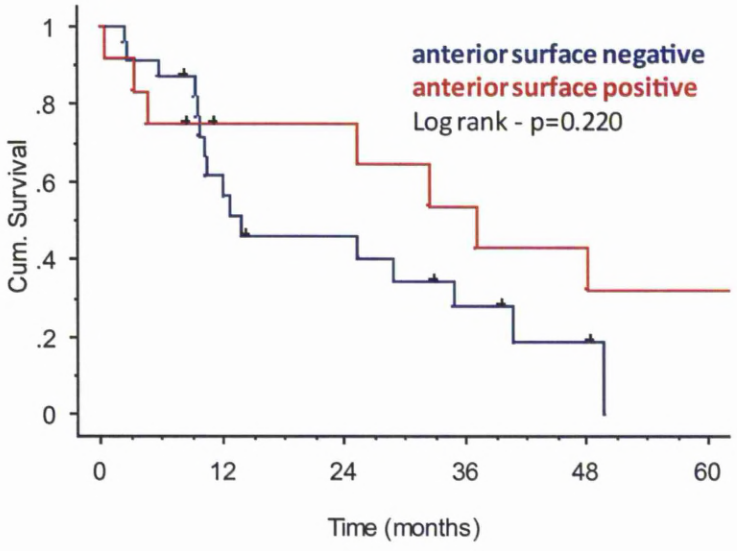
No. at risk						
CRT	6	3	2	0	0	0
No treatment	115	55	23	11	8	7

fig.17A - Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients stratified by lymph node 16b status (n=87).



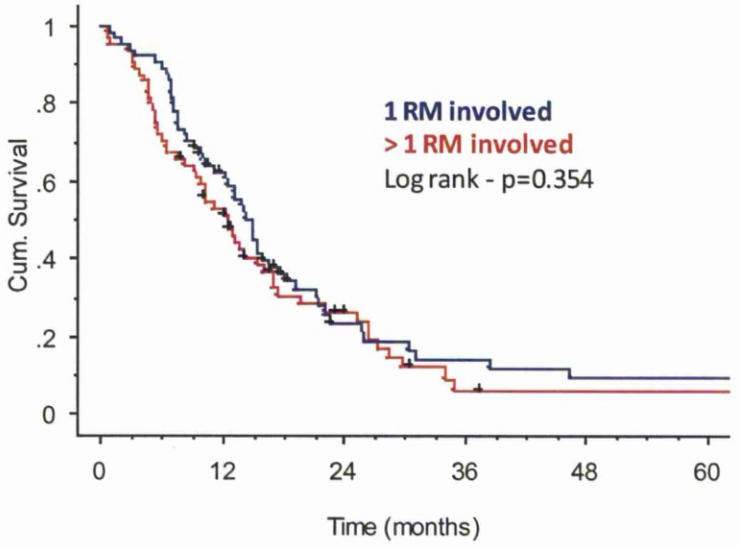
No. at risk						
LN16b -ve	76	44	23	11	7	5
LN16b +ve	11	6	2	1	0	0

fig.21A - Kaplan-Meier cumulative survival curves for R0 cases stratified according to the presence of tumour involvement (direct or < 1mm) of the anterior pancreatic surface (n=35).



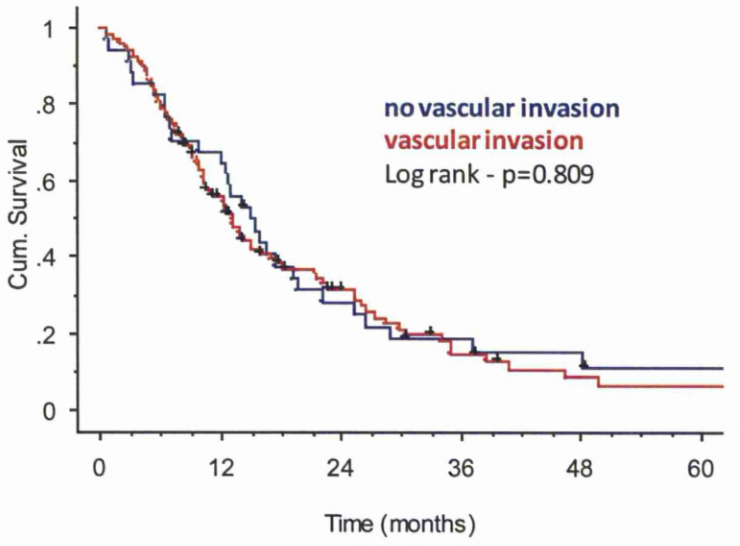
No. at risk						
anterior -ve	23	12	8	4	2	0
anterior +ve	12	7	7	5	4	3

fig.23A - Kaplan-Meier cumulative survival curves for R1 resections stratified according to single vs. multifocal resection margin involvement (n=128).



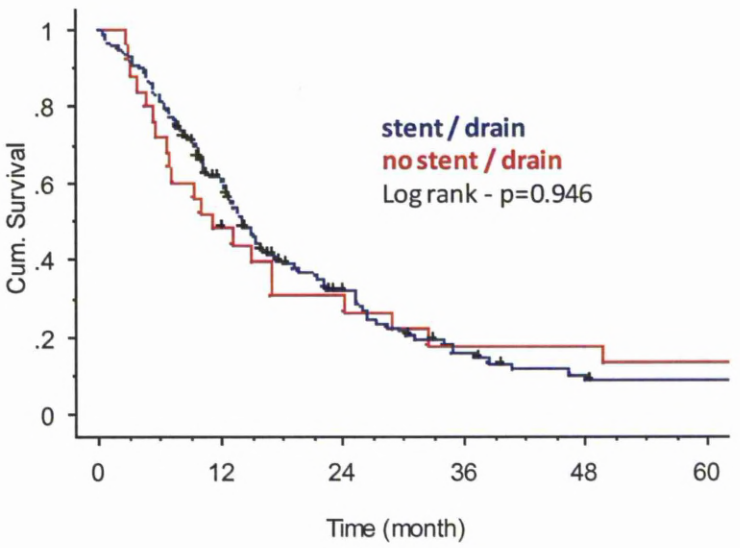
No. at risk						
1 RM	64	36	10	6	4	4
>1 RM	64	32	12	2	1	1

fig.26A - Kaplan-Meier cumulative survival curves for resected pancreatic ductal adenocarcinoma cases stratified according to the presence of vascular invasion (n=138).



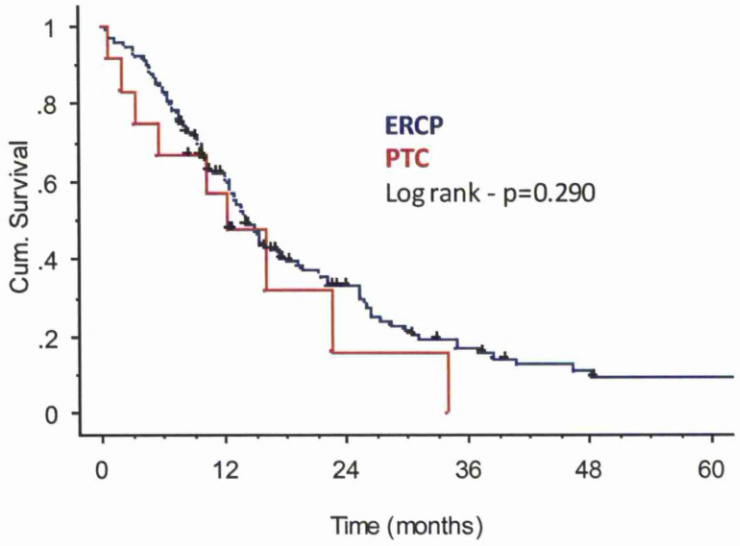
No. at risk						
no vasc. inv.	34	23	9	5	4	2
vasc. invasion	104	50	22	9	4	3

fig.27A - Kaplan-Meier cumulative survival curves according to preoperative biliary drainage (n=166).



No. at risk						
stent/drain	141	76	31	13	7	5
none	25	12	7	4	4	3

fig.28A - Kaplan-Meier cumulative survival curves according to endoscopic vs. percutaneous drainage (n=141).



No. at risk						
ERCP	129	70	30	13	7	5
PTC	12	6	1	0	0	0

Table 2A - Standardised data extraction form

Survival analysis	**staining positive**	**staining negative**
Number of patients analysed:		
Number of patients excluded from analysis:		
Overall / disease-free / progression-free survival:		
Observed events in group:		
Number of censored cases in each group:		
Survival advantage to which group:		
Kaplan-Meier curves shown:		
Numbers at risk quoted:		
Median survival times quoted:		
Univariate test statistic quoted:		
Univariate hazard ratio (+/- 95% CI) quoted:		
Min. / Max. follow-up period:		
Recruitment period:		
Median follow-up period quoted:		
Correlation with clinico-pathological data	**staining positive**	**staining negative**
Male / female:		
Median age:		
T stage: 1/2 3/4		
Differentiation: Well Moderate poor		
Nodal status: Positive Negative		
Margin status: Positive Negative		
Stage: I II III IV		
Details of adjuvant therapy recorded:		

APPENDIX B - *Published papers*

Preoperative resolution of jaundice following biliary stenting predicts more favourable early survival in resected pancreatic ductal adenocarcinoma. Smith RA, Dajani K, Dodd S, Whelan P, Raraty M, Sutton R, Campbell F, Neoptolemos JP, Ghaneh P. *Ann Surg Oncol* 2008; 15: 3138-46.

Preoperative CA19-9 levels and lymph node ratio are independent predictors of survival in patients with resected pancreatic ductal adenocarcinoma. Smith RA, Bosonnet L, Ghaneh P, Raraty M, Sutton R, Campbell F, Neoptolemos JP. *Dig Surg* 2008; 25: 226-32.

The platelet-lymphocyte ratio improves the predictive value of serum CA19-9 levels in determining patient selection for staging laparoscopy in suspected periampullary cancer. Smith RA, Bosonnet L, Ghaneh P, Sutton R, Evans J, Healey P, Garvey C, Hughes M, Raraty M, Campbell F, Neoptolemos JP. *Surgery* 2008; 143: 658-66.

Preoperative platelet-lymphocyte ratio is an independent significant prognostic marker in resected pancreatic ductal adenocarcinoma. Smith RA, Bosonnet L, Raraty M, Sutton R, Neoptolemos JP, Campbell F, Ghaneh P. *Am J Surg* 2009; 197: 466-72.

Classification of R1 resections for pancreatic cancer: the prognostic relevance of tumour involvement within 1 mm of a resection margin. Campbell F, Smith RA, Whelan P, Sutton R, Raraty M, Neoptolemos JP, Ghaneh P. *Histopathology* 2009; 55: 277-83.

Meta-analysis of immunohistochemical prognostic markers in resected pancreatic cancer. Smith RA, Tang J, Tudur-Smith C, Neoptolemos JP, Ghaneh P. *BJC* 2011; 104:1440-1451.

Annals of Surgical Oncology
DOI: 10.1245/s10434-008-0148-z

Preoperative Resolution of Jaundice Following Biliary Stenting Predicts More Favourable Early Survival in Resected Pancreatic Ductal Adenocarcinoma

Richard A. Smith, MRCS,[1] K. Dajani, MRCS,[1] S. Dodd, MSc,[2,3] P. Whelan, BSc,[1] M. Raraty, FRCS,[1] R. Sutton, FRCS,[1] F. Campbell, FRCPath,[4] J. P. Neoptolemos, FRCS,[1,2] and P. Ghaneh, FRCS[1,2]

[1]Division of Surgery and Oncology, School of Cancer Studies, Royal Liverpool University Hospital, 5th Floor UCD Building, Daulby St, Liverpool L69 3GA, UK
[2]Cancer Research UK Liverpool Cancer Trials Unit, Cancer Research Centre, University of Liverpool, 200 London Rd, Liverpool L3 9TA, UK
[3]Centre for Medical Statistics and Health Evaluation, Faculty of Medicine, University of Liverpool, Shelley's Cottage, Brownlow St, Liverpool L69 3GS, UK
[4]Department of Pathology, Royal Liverpool University Hospital, Prescot St, Liverpool L7 8XP, UK

Introduction: Despite the widespread use of endoscopic biliary stenting in patients presenting with potentially resectable pancreatic cancer, there is no general consensus regarding whether this represents a superior management approach over expeditious surgical intervention. The objective of this study was to investigate the influence of preoperative biliary stenting and resolution of jaundice on subsequent postoperative survival following resection for pancreatic cancer.

Methods: 155 patients undergoing partial pancreatoduodenectomy for pancreatic ductal adenocarcinoma between January 1997 and August 2007 were identified from a prospectively maintained database.

Results: There was no survival difference when comparing patients undergoing preoperative biliary drainage ($n = 130$) with those who did not ($n = 25$) (log rank, $P = 0.981$). When analysing individual prognostic factors as continuous variables in univariate Cox analysis, lower albumin levels ($P = 0.016$), elevated alkaline phosphatase levels ($P = 0.011$) and elevated CRP levels ($P = 0.021$) were associated with poorer overall survival. Multivariable Cox regression demonstrated that both albumin ($P = 0.008$) and CRP ($P = 0.038$) remained significant independent predictors of overall survival alongside lymph node ratio ($P = 0.018$). Although preoperative bilirubin levels were not associated with overall survival when analysed as a continuous variable (Cox, $P = 0.786$), the presence of jaundice (i.e., bilirubin >35 μmol/l) at the time of surgery was a significant adverse predictor of early survival in patients undergoing preoperative biliary drainage (Breslow–Gehan–Wilcoxon, $P = 0.013$) and remained a significant predictor of early survival when included in a further Cox analysis with censoring of cases who survived beyond 6 months (Cox, $P = 0.017$).

Conclusion: These results suggest that the presence of jaundice at the time of resection has an adverse impact on early, but not overall, postoperative survival in pancreatic cancer patients undergoing preoperative biliary drainage.

Address correspondence and reprint requests to: Richard A. Smith, MRCS; E-mail: rsmith3@liverpool.ac.uk

Pancreatic ductal adenocarcinoma represents a major cause of cancer mortality in developed countries[1,2] and surgical resection remains the only

potentially curative intervention.[3] Since the advent of partial pancreatoduodenectomy, the preoperative status of patients undergoing pancreatic surgery has been well recognised as an important factor in determining postoperative outcome. The rationale originally described for pancreatoduodenectomy being conceived as a two-stage procedure, comprising cholecystogastrostomy and gastrojejunostomy followed by subsequent pancreatic resection, was based on optimising the overall physical status of the patient prior to definitive resection.[4]

Over 70 years later, the issue of how best to optimise patients with potentially resectable pancreatic and periampullary malignancy prior to definitive surgery remains entirely relevant. Despite the widespread use of endoscopic preoperative biliary stenting in this setting, there remains no conclusive evidence to prove that intervention to ameliorate obstructive jaundice prior to resection represents a superior management approach over expeditious surgery. The results of a previous meta-analysis suggest that preoperative biliary instrumentation is associated with an increased risk of perioperative morbidity over surgery alone (57% versus 42% respectively). However, this study failed to demonstrate any significant effect on operative mortality rates.[5] Although several previous studies have investigated the effect of preoperative biliary drainage on initial postoperative outcomes in pancreatic and periampullary cancer (e.g., 30-day mortality, duration of postoperative admission, ITU stay, in-patient complication rates, relaparotomy rates, etc.),[6–8] no previous published studies have specifically investigated the relationship between preoperative liver function and subsequent postoperative survival in patients undergoing resection for pancreatic cancer.

The objective of this study was to evaluate the potential relationship between preoperative biliary drainage, resolution of jaundice and early postoperative survival in pancreatic cancer patients undergoing partial pancreatoduodenectomy. The prognostic relevance of preoperative serum albumin and C-reactive protein (CRP) levels was also investigated as part of this analysis to determine whether these parameters represent potential confounding factors in describing the relationship between stenting, jaundice and survival.

METHODS

Consecutive patients undergoing partial pancreatoduodenectomy for histologically confirmed pancreatic ductal adenocarcinoma between January 1997 and August 2007 were identified from a prospective database. Histopathology reporting was undertaken according to the Royal College of Pathologists guidelines.[9] The lymph node ratio (i.e., number of tumour-involved lymph nodes as a ratio of total number of nodes sampled) was used in the survival analyses as this has been shown to be a superior prognostic variable when compared with overall nodal status (i.e., positive versus negative).[10]

Where patients underwent more than one procedure prior to successful stenting, the date of definitive biliary drainage was used for analysis. All patients with potentially resectable pancreatic tumours and obstructive jaundice presenting to our institution during the study period routinely underwent biliary decompression at endoscopic retrograde cholangiopancreatography (ERCP) where possible. Those in whom endoscopic drainage was unsuccessful went on to undergo percutaneous transhepatic cholangiography (PTC) or combined procedures with internal stenting. External drainage was required in a proportion of these cases (4/12). A plastic biliary endoprosthesis was routinely used. A metal stent was required in only 5 out of 126 cases undergoing internal drainage. Eleven of 25 unstented patients were jaundiced at the time of resection. Four of these 11 patients underwent failed endoscopic procedures and went straight to surgery without any additional percutaneous intervention. The remaining patients either developed jaundice between the time of initial presentation and admission for surgery or went straight to laparotomy on the basis of logistical reasons (i.e., early availability of theatre session). Jaundice was defined as a serum bilirubin concentration of >35 μmol/l (approximately 2 mg/dl). This level was selected as hyperbilirubinaemia is usually only clinically evident as jaundice when serum bilirubin levels exceed this value.

Statistical Analysis

Median, interquartile range (IQR) and 95% confidence intervals (CI) were used to describe continuous data and the two-tailed Mann–Whiney *U* test was used for comparative analyses. Overall survival times were calculated from the date of resection to the date of death. Kaplan–Meier curves were used to illustrate the survival trends for the prognostic variables of interest. The log rank (Cox–Mantel) test was used to assess the effect of prognostic factors on overall and late survival while the Breslow–Gehan–Wilcoxon test was used to assess the effect on early survival.[11] Cox proportional hazards regression was used for both

univariate and multivariable analyses. Proportionality was checked for all covariates prior to inclusion for Cox regression by checking the log cumulative hazard plots for each variable. The prognostic data were analysed using continuous variables for Cox regression where appropriate.[12] Only variables exhibiting significance on univariate analysis were included in the multivariable analyses. An additional Cox analysis was conducted with censoring of cases who survived >6 months in order to identify which prognostic factors were specifically associated with early, rather than overall, postoperative survival. For the analysis of the prognostic significance of CRP, patients with preoperative values >100 mg/l were excluded in order to minimise the confounding effect of acute cholangitis.

RESULTS

Six hundred twenty-three patients underwent surgical intervention for suspected pancreatic or periampullary cancer during the study period. Resections were performed in 432 patients, of whom 351 underwent pancreatoduodenectomy. One hundred fifty-five patients had histologically confirmed pancreatic ductal adenocarcinoma. Table 1 reports the demographics and proportion of patients undergoing preoperative biliary drainage in this group. There were 33 censored cases with a median follow-up time of 11.5 (IQR 6.2–23.1) months. The overall median survival was 13.3 (95% CI 11.1–15.5) months. The median time interval from the date of preoperative liver function tests to resection was 1 (IQR 1–2) day. The median time interval from the date of preoperative CRP estimation to resection was 2 (IQR 1–11) days. Forty-one patients (26%) received some form of adjuvant therapy following resection, of whom 35 received chemotherapy.

TABLE 1. *Patient demographics and details of preoperative biliary drainage*

No. of patients identified	155
Gender: male (%)	88 (56.8)
Age: median (IQR)	67 (61–72) years
No. (%) of cases with preoperative LFTs recorded:	151 (97.4)
Bilirubin: median (IQR)	25 (12–56) μmol/l
Alkaline phosphatase: median (IQR)	202 (122–344) U/l
ALT: median (IQR)	49 (26–81) U/l
γGT: median (IQR)	125 (65–352) U/l
Albumin: median (IQR)	36 (32–40) mg/l
No. of cases with preoperative CRP recorded:	121 (78.1)
CRP: median (IQR)	11 (5–26) mg/l
Intervention for preoperative biliary drainage* (%):	
None	25 (16.1)
ERCP + stent	118 (76.1)
PTC/combined procedure + stent/drain	12 (7.7)
Interval from stenting to surgery: median (IQR)	33 (20–49) days

LFTs, liver function tests; ALT, alanine aminotransferase; γGT, gamma-glutamyl transferase; IQR, interquartile range; CRP, C-reactive protein; ERCP, endoscopic retrograde cholangiopancreatography; PTC, percutaneous transhepatic cholangiopancreatography.

* Four cases undergoing PTC required external drainage. A metal biliary stent was used in 5 cases out of 126 cases undergoing internal stenting. A plastic stent was employed in the remaining cases.

Early Mortality

There were three postoperative deaths within 30 days of surgery (2%) and a total of six patients (4%) died prior to discharge from hospital. The cause of death in those patients who died prior to discharge was pneumonia in three cases, and myocardial infarction, multiple organ failure following relaparotomy for a bile leak, and intra-abdominal sepsis in the remaining cases. The 90-day mortality rate was 6% (10/155 patients). Of 149 patients followed up for a minimum of 6 months there were 30 recorded deaths within 6 months of resection (20%). Death within 6 months of surgery was used to define "early" survival in the subsequent Cox analysis.

The Effect of Preoperative Biliary Drainage on Survival

Figure 1A demonstrates that there was no significant difference in early or overall survival when comparing those patients who did or did not undergo biliary drainage preoperatively (log rank, $P = 0.981$). Figure 1B demonstrates no significant difference in survival when comparing patients who required percutaneous intervention for biliary drainage with those who underwent endoscopic stenting (log rank, $P = 0.230$).

Preoperative CRP levels were found to be more significantly elevated in those cases requiring PTC [median (IQR) = 29 (22–107) mg/l] when compared with those stented at ERCP [median (IQR) = 10 (4–23) mg/l] (Mann–Whitney, $P = 0.022$). There was no significant difference, however, in CRP levels at the time of surgery when comparing those cases who underwent endoscopic stenting with those cases who did not undergo any form of preoperative biliary drainage [median (IQR) = 10 (7–57) mg/l] (Mann–Whitney, $P = 0.487$).

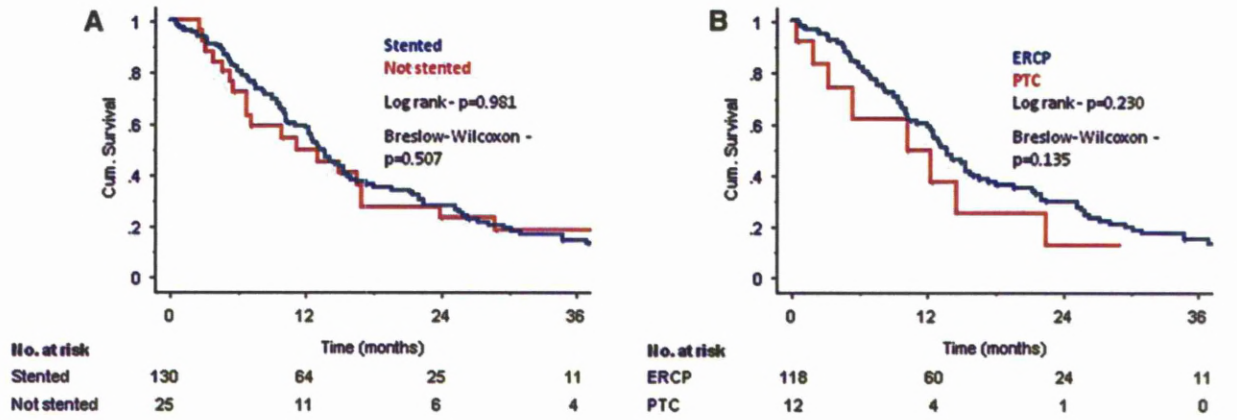


FIG. 1. Kaplan–Meier cumulative survival curves to demonstrate the effect of preoperative biliary stenting on survival following pancreatoduodenectomy for pancreatic ductal adenocarcinoma: (**A**) stented versus nonstented patients, and (**B**) patients stented at endoscopic retrograde cholangiopancreatography (ERCP) versus percutaneous transhepatic cholangiography (PTC).

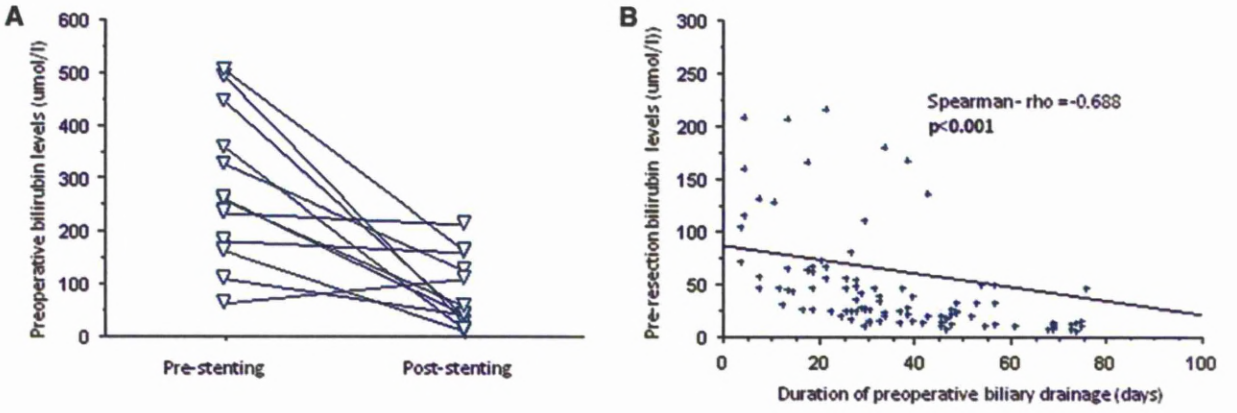


FIG. 2. (**A**) Bivariate line chart to illustrate pre- and post-stenting bilirubin levels in 12 patients requiring percutaneous intervention for preoperative biliary drainage. Each line signifies an individual patient's results. There was a median reduction in bilirubin levels of 73% (IQR 49–92%) in patients undergoing PTC. However, only three patients (25%) experienced complete resolution of jaundice (i.e., ≤35 μmol/l). (**B**) Scattergram to demonstrate the significant inverse correlation between duration of preoperative biliary drainage (i.e., time interval between stenting and resection) and bilirubin levels recorded at the time of surgery.

The Effect of Biliary Drainage on Resolution of Jaundice

Patients requiring PTC exhibited a greater degree of residual jaundice at the time of surgery [median bilirubin level 50 (IQR 34–134) μmol/l] when compared with patients stented at ERCP [median bilirubin level 24 (IQR 12–47) μmol/l] (Mann–Whitney, $P = 0.041$). Figure 2A illustrates the change in preoperative bilirubin levels before and after biliary decompression in patients undergoing PTC ($n = 12$). There was a median reduction in bilirubin levels of 73% (IQR 49–92%) in patients requiring PTC or combined procedures. However, only 3 of 12 patients (25%) experienced complete resolution of jaundice (i.e., ≤35 μmol/l) at the time of surgery.

When analysing patients stented endoscopically, there was a median reduction in preoperative bilirubin levels of 85% (IQR 74–93%) for these patients. In total, 64% (73/114) of patients undergoing stenting at ERCP had complete resolution of jaundice at the time of surgery.

Duration of Preoperative Biliary Drainage and Resolution of Jaundice

The median time interval between definitive biliary drainage and surgery was 36 (IQR 25–53) days in

TABLE 2. *Multivariable Cox proportional hazards analysis of preoperative CRP and albumin as predictors of overall survival.*

	Cox model to evaluate factors predictive of overall survival					
	Univariate analysis			Multivariable analysis (n = 107)		
	Hazard ratio (95% CI)	χ^2	P	Hazard ratio (95% CI)	χ^2	P
Bilirubin:						
(Continuous)	1.000 (0.999–1.001)	0.074	0.786	–	–	–
>35 μmol/l	1.338 (0.929–1.927)	2.443	0.118	–	–	–
Albumin	0.963 (0.935–0.993)	5.800	**0.016**	0.933 (0.887–0.982)	6.976	**0.008**
Alkaline phosphatase	1.001 (1.000–1.002)	6.538	**0.011**	1.000 (0.999–1.001)	0.114	0.736
CRP	1.011 (1.002–1.020)	5.323	**0.021**	1.012 (1.001–1.024)	4.288	**0.038**
Tumour size	1.022 (1.007–1.038)	8.208	**0.004**	1.015 (0.995–1.035)	2.227	0.136
Poor tumour differentiation*	1.615 (1.112–2.345)	6.327	**0.012**	1.415 (0.831–2.408)	1.636	0.201
Lymph node ratio	3.713 (1.586–8.692)	9.144	**0.003**	4.212 (1.281–13.856)	5.604	**0.018**
Resection margin positive	1.594 (1.055–2.406)	4.915	**0.027**	1.167 (0.636–2.144)	0.250	0.617
Adjuvant chemotherapy	0.548 (0.342–0.878)	6.260	**0.012**	0.666 (0.383–1.158)	2.227	0.150

* Poor tumour differentiation analysed against well/moderately differentiated tumours. Resection margin status (R1 versus R0) and adjuvant chemotherapy (yes versus no) were also analysed as categorical covariates. Albumin, alkaline phosphatase, CRP, tumour size and lymph node ratio were included as continuous covariates in the Cox model.

Note: hazard ratios for continuous prognostic data reflect increase in relative hazard with each unit increase in covariate value.

Statistically significant P-values (i.e., those <0.050) were highlighted in bold.

patients stented endoscopically. This time interval was 26 (IQR 14–37) days in patients requiring PTC or combined procedures (Mann–Whitney, $P = 0.128$). When analysing the overall group of patients undergoing preoperative biliary drainage, there was a significant inverse correlation between the duration of biliary drainage and bilirubin levels prior to resection (Spearman rho = –0.688, $P < 0.001$); i.e., a longer period of biliary drainage resulted in lower bilirubin levels at the time of surgery (Fig. 2B).

The Influence of Preoperative Liver Function and CRP on Overall Survival

Table 2 demonstrates the results of univariate Cox proportional hazards regression when modelling the various preoperative blood results as continuous prognostic variables in the overall patient group undergoing resection for pancreatic cancer. The hazard ratios quoted for each variable reflect the increase in the relative hazard of death associated with each unit increase in the prognostic variable.

The prognostic value of preoperative CRP in the overall patient group for whom levels were recorded was not significant ($n = 121$; $P = 0.309$). However, this was found to be significant when excluding a small number ($n = 8$) of outlying cases with preoperative CRP levels >100 mg/l ($n = 113$; $P = 0.021$). Figure 3A illustrates this relationship when using a cutoff value of 10 mg/l (this value was selected according to the normal laboratory reference range for CRP). The eight excluded patients with CRP levels >100 mg/l all underwent intervention for biliary drainage; the median preoperative bilirubin level recorded for these patients was 140 (IQR 64–249) μmol/l, the median alkaline phosphatase level was 232 (168–534) U/l and the median WBC was 13.2 (10.3–15.3) $\times 10^9$/l. The median survival for this excluded group was 13.3 months.

Univariate Cox analysis demonstrated a significant inverse association between preoperative albumin levels and postoperative survival ($P = 0.016$) while elevated alkaline phosphatase levels were also associated with a significant trend towards poorer postoperative survival ($P = 0.011$). Figure 3B and C show the results of the Kaplan–Meier analyses to illustrate these survival trends. Preoperative alanine aminotransferase ($P = 0.807$), γ-glutamyl transferase ($P = 0.233$) and prothrombin time ($P = 0.822$) failed to exhibit any association with overall survival.

Table 2 also shows the results of a multivariable Cox proportional hazards analysis including preoperative CRP, albumin and alkaline phosphatase levels as prognostic variables alongside the resected histological tumour characteristics of significance. CRP ($P = 0.038$) and albumin ($P = 0.008$) were found to maintain significance on multivariable analysis alongside the lymph node ratio ($P = 0.018$).

The Influence of Preoperative Bilirubin Levels on Early Postoperative Survival in Resected Pancreatic Cancer Patients Undergoing Biliary Stenting

Although preoperative bilirubin levels were not found to exhibit a significant relationship with *overall* survival when modelled as a continuous variable

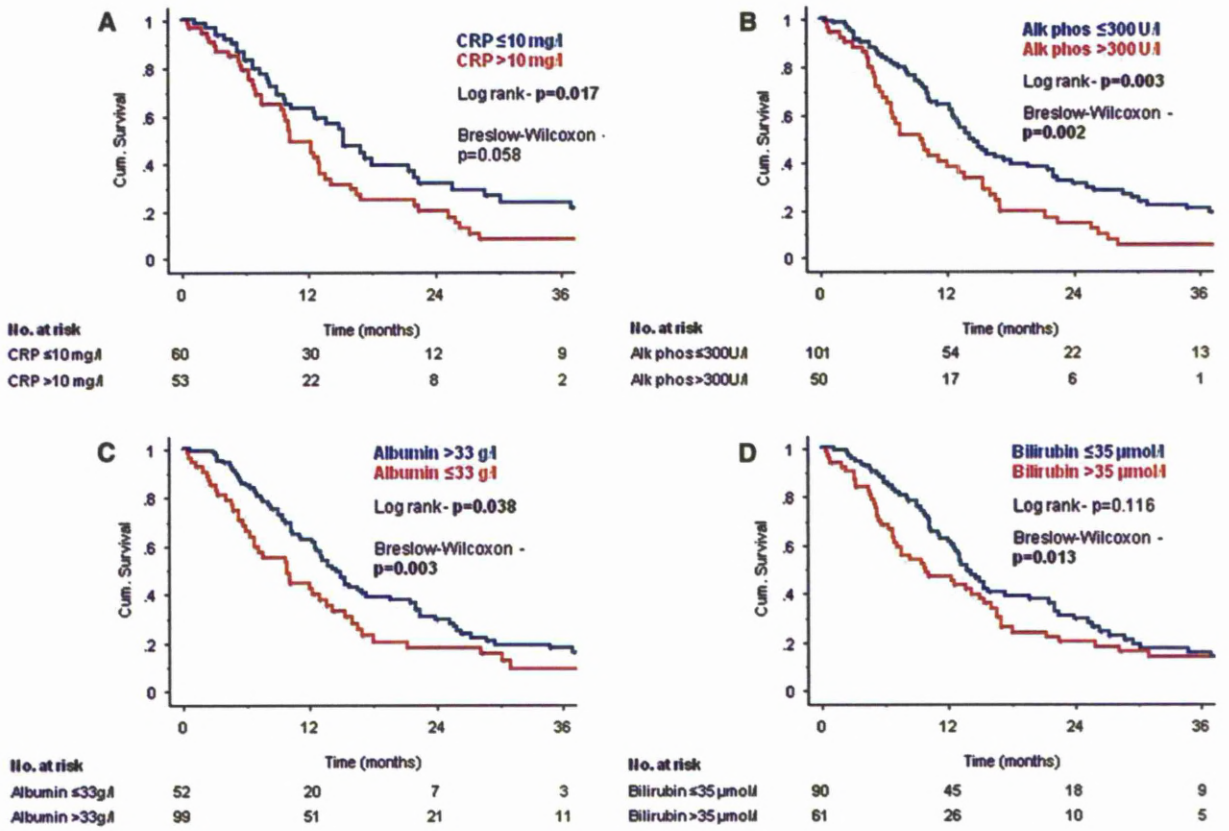


FIG. 3. Kaplan–Meier cumulative survival curves according to preoperative C-reactive protein (**A**), alkaline phosphatase (**B**), albumin (**C**) and bilirubin (**D**).

(Table 2), Fig. 3D demonstrates a clear trend towards less favourable *early* survival in jaundiced patients (i.e., bilirubin levels >35 µmol/l) at the time of resection (Breslow–Gehan–Wilcoxon, $P = 0.013$). An additional multivariable analysis was undertaken in order to identify whether this observed relationship between resolution of jaundice and early survival was independent of other factors. A second Cox model was used to investigate which prognostic variables were specifically associated with 6-month survival (Table 3). Only patients undergoing preoperative biliary drainage were included in this analysis ($n = 130$). All cases who survived beyond 6 months ($n = 100$) were censored at 6 months for the purposes of this analysis. The results from this additional Cox model suggest that the association between residual jaundice at the time of resection and early survival was independent of other prognostic factors. CRP failed to exhibit any effect on early survival even when excluding cases with levels >100 mg/l ($P = 0.731$). This trend was also evident from Fig. 2A. There were no clinicopathological differences between stented patients who did or did not experience resolution of jaundice at the time of resection (data not shown).

DISCUSSION

There is currently no general consensus regarding whether preoperative biliary drainage prior to surgical intervention represents the optimal management approach in patients presenting with potentially resectable pancreatic cancer. A meta-analysis has suggested that preoperative intervention for biliary drainage is associated with an increased risk of early postoperative morbidity (principally relating to wound infection). However, no overall association between biliary drainage and perioperative mortality was identified in this study.[5] This meta-analysis was based on level 1 evidence from five randomised trials comprising 302 periampullary cancers in total.[13–17] Less than half of these patients had pancreatic adenocarcinoma and a similar proportion of the overall group actually underwent resection. These five trials

TABLE 3. *Multivariable Cox proportional hazards analysis of preoperative jaundice (i.e., bilirubin >35 μmol/l) as a predictor of early survival in cases undergoing preoperative biliary drainage*

	Cox model to evaluate factors predictive of early survival					
	Univariate Cox analysis			Multivariable Cox analysis (n = 120)		
	Hazard ratio (95% CI)	χ^2	P	Hazard ratio (95% CI)	χ^2	P
Bilirubin >35 μmol/l	5.058 (1.993–12.840)	11.635	**<0.001**	3.319 (1.245–8.849)	5.748	**0.017**
Albumin*	0.897 (0.842–0.955)	11.382	**<0.001**	0.913 (0.837–0.997)	4.110	**0.043**
Alkaline phosphatase*	1.001 (1.000–1.002)	3.161	0.075	–	–	–
CRP*	0.999 (0.987–1.011)	0.035	0.852	–	–	–
Tumour size*	1.040 (1.008–1.073)	6.083	**0.014**	1.037 (1.004–1.072)	4.783	**0.029**
Poor tumour differentiation	2.271 (1.002–5.150)	3.858	**0.049**	1.974 (0.814–4.790)	2.264	0.132
Lymph node ratio*	3.111 (0.416–23.273)	1.222	0.269	–	–	–
Resection margin positive	1.389 (0.548–3.524)	0.479	0.489	–	–	–
Adjuvant chemotherapy	0.124 (0.017–0.919)	4.175	**0.041**	0.202 (0.027–1.538)	2.385	0.123

* Albumin, alkaline phosphatase, CRP, tumour size and lymph node ratio were analysed as continuous covariates. Resection margin status (R1 versus R0) and adjuvant chemotherapy (yes versus no) were also analysed as categorical covariates. There were 22 deaths within 6 months of surgery in the 120 patients included in the multivariate Cox model. Patients who survived >6 months were right-censored in this Cox analysis in order to describe the relationship between prognostic covariates and early survival.

Statistically significant P-values (i.e., those <0.050) were highlighted in bold.

encompassed a mix of both endoscopic and percutaneous procedures and included cases undergoing both internal and external drainage. The median bilirubin level at the time of surgery for the pooled patient group undergoing preoperative biliary drainage was recorded as 157 μmol/l. This represents a significantly greater value than the result recorded in the present study (25 μmol/l). Given the mix of periampullary tumours and the inclusion of both resected and unresected cases in the above meta-analysis, the overall findings from this study might not be reliably extrapolated to the specific setting of resected pancreatic cancer. Nevertheless, the results of the present study are concordant with the above findings in that biliary stenting per se was not shown to have any adverse effect on early or late survival in the overall patient group undergoing partial pancreatoduodenectomy for pancreatic cancer. Given the limited number of unstented patients in this analysis, the negative finding from this analysis may be the result of inadequate sample size.

No analysis of postoperative morbidity was undertaken in the present study as the association between preoperative biliary drainage and perioperative complications has been extensively evaluated elsewhere.[5–8] The relationship between preoperative jaundice and cancer-specific versus operative-related mortality could not be analysed in this study as insufficient clinical information was available to make a reliable retrospective assessment of cause of death in those patients who died within 6 months of surgery following discharge from hospital.

The principal finding of this study was the observed association between residual jaundice at the time of surgery and less favourable early survival. A recent abstract has also suggested that preoperative liver function may represent a significant predictor of survival in resected pancreatic cancer.[18] In the present study, separation of the survival curves was only evident within the first 12 postoperative months resulting in a significant P-value when using the Breslow–Gehan–Wilcoxon test, but not the log rank (Cox–Mantel) test. The Breslow–Gehan–Wilcoxon test is calculated according to the number of patients at risk along each point of the survival curve and provides a better discriminator of differences in early survival between two groups—the follow-up period during which the majority of patients are still alive.[11] In comparison, the log rank test, which is calculated according to equal weighting at each point along the survival curve, provides a better indication of differences in overall and late survival.

The relationship between jaundice at the time of surgery and early survival in patients undergoing preoperative biliary drainage was further investigated by generating a conditional Cox regression model where all events occurring after a specified time period (i.e., 6 months) were considered as censored in order to analyse the effect of multiple covariates on early survival. The process of "fixed right-censoring" is analogous to "end-of-study censoring" where a study is designed in such a way as to assess survival only within a specified follow-up period. Events occurring after this are censored at the point of the maximum follow-up period (as per the Cox model used in the present study). This type of fixed censoring is noninformative, allowing use of standard likelihood-based statistical approaches (i.e., log rank, Cox regression,

etc.) for comparative analyses of survival within a specified follow-up period.[19] The results from this analysis indicate that the adverse association between residual jaundice at the time of surgery and early postoperative survival was independent of hypoalbuminaemia and histological parameters.

The results demonstrate that the majority of patients with resectable pancreatic cancer who present with obstructive jaundice can undergo successful preoperative biliary drainage at ERCP. The median bilirubin level at the time of surgery was 24 μmol/l in this patient group, reflecting an 85% median reduction in bilirubin levels from presentation. Of the small proportion of patients who could not be stented endoscopically who went on to undergo percutaneous drainage, these patients also had a significant reduction in bilirubin levels. However, despite more invasive intervention, these patients were still less likely to experience resolution of jaundice at the time of resection. The results also demonstrate that a longer period of preoperative biliary drainage was inversely correlated with bilirubin levels at the time of surgery. These findings suggest that a balance exists with regard to the optimal timing of definitive surgery for this patient group, in order to allow resolution of jaundice where possible without compromising the window of opportunity for tumour resectability. This issue is particularly relevant for patients where borderline features of resectability are present on initial imaging or where resolution of jaundice is protracted, even following percutaneous intervention. The decision-making process regarding the optimal timing of surgery is clearly a multifactorial one which may need to incorporate a number of additional logistical issues and should be considered on an individual patient basis. However, the observations from the present study indicate that early survival outcomes may be adversely influenced by inadequate resolution of preoperative jaundice.

Elevated CRP levels were found to be associated with overall but not early survival when excluding patients with preoperative CRP values >100 mg/l. Cholangitis represents the most common cause for acute sepsis preoperatively in patients undergoing biliary decompression and stenting has also been shown to be associated with positive bile cultures at laparotomy.[20,21] Several studies have demonstrated that pre-resection CRP levels represent a potential prognostic factor in other gastrointestinal malignancies.[22–24] In the single previous study in resected pancreatic cancer ($n = 65$), an adverse association between elevated preoperative CRP and survival was shown on univariate but not multivariate analysis.[25]

The results from the present study demonstrate that the relationship between elevated preoperative CRP levels and poorer survival is only evident when excluding a small number of outlying patients with a significantly elevated CRP. It was not possible to determine which patients in this study had preoperative clinical features of cholangitis on a retrospective basis and the selection of a CRP cutoff value of 100 mg/l for exclusion was a pragmatic one rather than being based on any predefined diagnostic criteria. However, when analysing the eight excluded patients with CRP levels >100 mg/l, this subgroup of patients all underwent preoperative intervention for biliary drainage and had biochemical findings consistent with biliary sepsis at the time of CRP estimation. It was therefore felt to be reasonable to conclude that the disparity in the survival analyses outlined above was principally due to the confounding effect of cholangitis in the group of eight patients with significantly elevated CRP levels. The median survival of these eight excluded patients was also found to be comparable with the overall patient group, indicating that the exclusion of these patients from the Cox analysis would be unlikely to have a significant effect in skewing the overall survival results.

Although there was no significant difference between preoperative CRP levels in patients requiring endoscopic biliary drainage when compared with unstented patients, percutaneous intervention was associated with higher CRP levels at the time of surgery. Percutaneous access to the biliary tree is clearly a more invasive route than that associated with ERCP. Furthermore, patients who required PTC or combined procedures would have already undergone one or more unsuccessful attempts at endoscopic biliary stenting. Therefore, the association between elevated CRP levels and PTC is likely to reflect the increased tissue damage associated with percutaneous access in addition to increasing the degree of bactobilia and consequent likelihood of biliary sepsis due to the cumulative effect of previous endoscopic biliary instrumentation prior to successful drainage. Our results suggest that, while elevated preoperative CRP levels exhibit an independent association with overall survival, the presence of cholangitis and the requirement for percutaneous biliary decompression are likely to represent significant confounding factors when interpreting the prognostic value of preoperative CRP levels in this patient group.

Preoperative albumin levels were found to be a significant predictor of both early and overall survival in the present study. Although previous studies have demonstrated that hypoalbuminaemia is associated

with less favourable survival outcomes in patients with inoperable pancreatic cancer[26] and higher morbidity and mortality rates following partial pancreatoduodenectomy,[27] no previous study has shown that pre-resection albumin levels are also associated with overall postoperative survival following resection for pancreatic cancer.[25] The causative mechanism for this association is unclear but impaired liver function and nutritional status at the time of surgery may be implicated. A recent study has suggested that the introduction of immuno-enriched nutritional supplements in the preoperative setting may yield significant improvements in early postoperative outcomes for patients undergoing major pancreatic resections.[28]

A multicentre randomised controlled trial which specifically aims to answer the question of whether preoperative biliary drainage or prompt surgical intervention represents the optimal management approach in potentially resectable pancreatic and periampullary cancer is currently being conducted.[29] The results of the present study suggest that the presence of jaundice at the time of pancreatic resection has an adverse impact on early, but not overall, postoperative survival in resected pancreatic cancer patients undergoing preoperative biliary drainage.

ACKNOWLEDGEMENT

Financial support—Cancer Research UK (Registered Charity No. 1089464)

REFERENCES

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. *CA Cancer J Clin* 2007; 57:43–66.
2. International Association of Cancer Registries. *Cancer Mondial*. http://www-dep.iarc.fr/.
3. Alexakis N, Halloran C, Raraty M, et al. Current standards of surgery for pancreatic cancer. *Br J Surg* 2004; 91:1410–27.
4. Whipple AO, Parsons WB, Mullins CR. Treatment of carcinoma of the ampulla of Vater. *Ann Surg* 1935; 102:763–79.
5. Sewnath ME, Karsten TM, Prins MH, et al. A meta-analysis on the efficacy of preoperative biliary drainage for tumors causing obstructive jaundice. *Ann Surg* 2002; 236:17–27.
6. Povoski SP, Karpeh MS, Conlon KC, et al. Association of preoperative biliary drainage with postoperative outcome following pancreaticoduodenectomy. *Ann Surg* 1999; 2:131–42.
7. Pisters PW, Hudec WA, Hess KR, et al. Effect of preoperative biliary decompression on pancreatiocduodenectomy-associated morbidity in 300 consecutive patients. *Ann Surg* 2001; 234:47–55.
8. Sewnath ME, Birjmohun RS, Rauws EAJ, et al. The effect of preoperative biliary drainage on postoperative complications after pancreaticoduodenectomy. *J Am Coll Surg* 2001; 192:726–34.
9. Campbell F, Bennett M, Foulis AJ. Minimum Dataset for Histopathological Reporting of Pancreatic, Ampulla of Vater and Bile Duct Carcinoma. *Royal College of Pathologists*; 2002. http://www.rcpath.org.
10. Pawlik TM, Gleisner AL, Cameron JL, et al. Prognostic relevance of lymph node ratio following pancreaticoduodenectomy for pancreatic cancer. *Surgery* 2007; 141:610–8.
11. Gehan EA. A generalised Wilcoxon test for comparing arbitrarily singly-censored samples. *Biometrica* 1965; 52:203–23.
12. Altman DG, Lausen B, Sauerbrei W, et al. Dangers of using optimal cutpoints in the evaluation of prognostic factors. *J Nat Cancer Inst* 1994; 86:829–35.
13. Hatfield AR, Tobias R, Terblanche J, et al. Preoperative external biliary drainage in obstructive jaundice. A prospective controlled clinical trial. *Lancet* 1982; 2:896–9.
14. McPherson GA, Benjamin IS, Hodgson HJ, et al. Preoperative percutaneous transhepatic biliary drainage: the results of a controlled trial. *Br J Surg* 1984; 71:371–5.
15. Pitt HA, Gomes AS, Lois JF, et al. Does preoperative percutaneous drainage reduce operative risk or increase hospital cost? *Ann Surg* 1985; 201:545–53.
16. Smith RC, Pooley M, George CR, et al. Preoperative percutaneous transhepatic internal drainage in obstructive jaundice: a randomized, controlled trial examining renal function. *Surgery* 1985; 97:641–8.
17. Lai EC, Mok FP, Fan ST, et al. Preoperative endoscopic drainage for malignant obstructive jaundice. *Br J Surg* 1994; 81:1195–8.
18. Tsao RE, Almhanna K, Lazaryan A, et al. Is adjuvant radiation plus chemotherapy for resectable pancreatic adenocarcinoma associated with improved survival? The Cleveland Clinic experience. ASCO 2008. Abstract No. 201.
19. Leung K, Elashoff RM, Abdelmonem AA. Censoring issues in survival analysis. *Annu Rev Public Health* 1997; 18:83–104.
20. Jagannath P, Dhir V, Shrikhande S, et al. Effect of preoperative biliary stenting on immediate outcome after pancreaticoduodenectomy. *Br J Surg* 2005; 92:356–61.
21. Jethwa P, Breuning E, Bhati C, et al. The microbiological impact of pre-operative biliary drainage on patients undergoing hepato-biliary-pancreatic (HPB) surgery. *Aliment Pharmacol Ther* 2007; 25:1175–80.
22. Nozoe T, Saeki H, Sugimachi K. Significance of preoperative elevation of serum C-reactive protein as an indicator of prognosis in esophageal carcinoma. *Am J Surg* 2001; 182:197–201.
23. Hashimoto K, Ikeda Y, Korenaga D, et al. The impact of preoperative serum C-reactive protein on the prognosis of patients with hepatocellular carcinoma. *Cancer* 2005; 103:1856–64.
24. Crozier JE, McKee RF, McArdle CS, et al. Preoperative but not postoperative systemic inflammatory response correlates with survival in colorectal cancer. *Br J Surg* 2007; 94:1028–32.
25. Jamieson NB, Glen P, McMillan DC, et al. Systemic inflammatory response predicts outcome in patients undergoing resection for ductal adenocarcinoma head of pancreas. *Br J Cancer* 2005; 17:21–3.
26. Glen P, Jamieson NB, McMillan DC, et al. Evaluation of an inflammation-based prognostic score in patients with inoperable pancreatic cancer. *Pancreatology* 2006; 6:450–3.
27. Winter JM, Cameron JL, Yeo CJ, et al. Biochemical markers predict morbidity and mortality after pancreaticoduodenectomy. *J Am Coll Surg* 2007; 204:1029–36.
28. Giger U, BüchlerM , Farhadi J, et al. Preoperative immunonutrition suppresses perioperative inflammatory response in patients with major abdominal surgery-a randomized controlled pilot study. *Ann Surg Oncol* 2007; 14:2798–806.
29. van der Gaag NA, de Castro SM, Rauws EA, et al. Preoperative biliary drainage for periampullary tumors causing obstructive jaundice; Drainage vs (direct) Operation (DROP-trial). *BMC Surg* 2007; 7:3.

Original Paper

Digestive Surgery

Dig Surg 2008;25:226–232
DOI: 10.1159/000140961

Received: January 16, 2008
Accepted: February 29, 2008
Published online: June 24, 2008

Preoperative CA19-9 Levels and Lymph Node Ratio Are Independent Predictors of Survival in Patients with Resected Pancreatic Ductal Adenocarcinoma

A. Smith[a] L. Bosonnet[a] P. Ghaneh[a] M. Raraty[a] R. Sutton[a] F. Campbell[b]
P. Neoptolemos[a]

[a]Division of Surgery and Oncology, School of Cancer Studies, University of Liverpool, and [b]Department of Pathology, Royal Liverpool University Hospital, Liverpool, UK

Key Words
Carbohydrate antigen · Pancreatic cancer · Prognostic factors

Abstract
Background: The aim of this study was to identify whether preoperative CA19-9 levels might represent an independent prognostic marker for overall survival in patients undergoing resection for pancreatic ductal adenocarcinoma, and to describe the relationship between CA19-9 and tumour histology. ***Methods:*** 109 patients who had a pancreatoduodenectomy for pancreatic ductal adenocarcinoma with recorded preoperative CA19-9 levels were identified from a prospectively maintained database (1997–2006). Multivariate analysis was conducted using a Cox proportional hazards model with continuous covariates where possible. ***Results:*** The median survival of 64 patients with a preoperative CA19-9 level >150 kU/l was 10.4 months while in 45 patients with a CA19-9 level ≤150 kU/l this was 22.1 months (corrected p = 0.012). Also significant on univariate analyses were overall lymph node status (p = 0.011), lymph node ratio (p = 0.003) and tumour diameter (p = 0.004). Preoperative CA19-9 levels >150 kU/l were associated with a larger, more poorly differentiated tumour along with an increased likelihood of positive resection margin status (all $p < 0.05$). Preoperative CA19-9 levels (p = 0.030) and lymph node ratio (p = 0.042) emerged as independent predictors of survival on multivariate analysis. ***Conclusions:*** Preoperative CA19-9 levels and lymph node ratio were significant predictors of survival in resected pancreatic ductal adenocarcinoma.

Introduction

The carbohydrate antigen 19-9 (CA19-9), a sialyated Lewis blood group antigen [1], is expressed in normal pancreatic ductal cells [2], and is also secreted in a mucin-bound form by the biliary and gallbladder mucosa and excreted in bile [3–5]. Obstructive jaundice will commonly precipitate elevated serum concentrations [6], and around 5% of the population are believed to lack the Lewis antigen glycosyltransferase enzyme required to synthesize CA19-9 [7]. A cut-off level of >37 kU/l is generally used as the optimal point at which pancreato-biliary malignancy can be differentiated from benign disease in symptomatic patients [8]. Although a CA19-9 cut-off value of >300 kU/l has previously been demonstrated to be required in order to diagnose biliary malignancy in the presence of concurrent cholestasis, CA19-9 levels for diagnostic purposes were not shown to be significantly affected by the presence of obstructive jaundice in cases of pancreatic malignancy [8].

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0253–4886/08/0253–0226$24.50/0

Accessible online at:
www.karger.com/dsu

Prof. J.P. Neoptolemos
The Owen and Ellen Evans Chair of Cancer Studies, Division of Surgery and Oncology, School of Cancer Studies, Royal Liverpool University Hospital
5th Floor UCD Building, Daulby St, Liverpool L69 3GA (UK)
Tel. +44 151 7064 175, Fax +44 151 7065 798, E-Mail j.p.neoptolemos@liverpool.ac.uk

Adopting a level of >150 kU/l targets a patient popula-
on for whom the diagnostic yield from staging laparos-
py is maximised in terms of detecting occult metastat-
disease [9]. One study demonstrated marked variability
the pattern of CA19-9 change following preoperative
solution of obstructive jaundice in cases of pancreatic
enocarcinoma, with levels falling in some cases and in-
easing in others [10]. Normalisation of CA19-9 levels
llowing resection for pancreatic cancer has been shown
be associated with a significant improvement in subse-
ent survival [11–13]. However, only a small number of
udies have investigated the potential role of preoperative
A19-9 levels in isolation as a prognostic index [14–16].

It was the objective of this study to investigate wheth-
CA19-9 levels might provide meaningful prognostic
formation prior to resection for pancreatic ductal ade-
carcinoma. In addition, we sought to investigate the
lationship between preoperative CA19-9 levels and his-
logical tumour characteristics and the extent to which
ncurrent cholestasis might act as a confounding factor.
ne prognostic value of the lymph node ratio was spe-
fically evaluated as this parameter has recently been
monstrated to represent a more powerful prognostic
arker than the overall nodal status in resected pancre-
ic cancer [17, 18]. Postoperative normalisation of CA19-
levels was also investigated for comparative purposes.

Patients and Methods

Consecutive pancreatic cancer patients undergoing pylorus-
eserving partial pancreatoduodenectomy or classical Kausch-
hipple resection between January 1997 and September 2006 at
e Royal Liverpool University Hospital were identified from a
ospectively maintained database. Only patients with histologi-
lly confirmed pancreatic ductal adenocarcinoma were included.
ata collected included patient demographics, operative details,
stological tumour characteristics including origin and classifi-
tion of the primary tumour, nodal status, tumour size, differen-
tion and resection margin status. A positive margin was defined
the presence of at least one cancer cell within 1 mm of one or
ore resection margins on microscopic examination. Pathology
porting was undertaken using the Royal College of Pathology
uidelines [19] according to the WHO classification [20] and
aged using the 6th edition of the UICC TNM system [21]. Details
preoperative intervention for biliary drainage, adjuvant therapy
ceived along with serum CA19-9 and bilirubin levels were also
corded. Survival data were obtained from hospital computer re-
rds, and date of last clinic attendance for censored cases.

Statistical Analyses

Median, interquartile range (IQR) and 95% confidence inter-
ls (CI) were used to describe the data. Continuous data were
alysed using the two-tailed Mann-Whitney U test with χ^2 and Fisher's exact for categorical data. Correlations between two continuous variables were analysed by Spearman's rank correlation. Survival data were analysed using the Kaplan-Meier method with corrected log-rank (Cox-Mantel) testing. Multivariate analysis was performed for variables with or approaching univariate significance using a Cox proportional hazards model with non-stepwise regression.

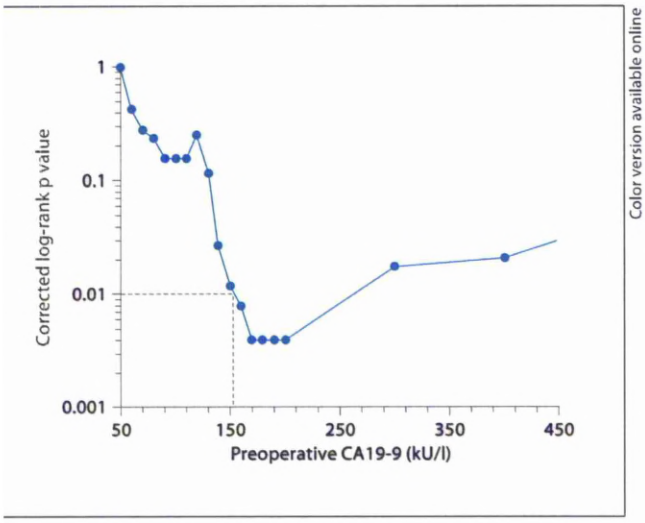
A corrected log-rank p value was used for a single 'optimal' cut-off value and parameters of interest were modelled as continuous covariates in a Cox model in order to obtain a more meaningful multivariate analysis [22]. This is because dichotomizing a continuous prognostic covariate can produce significant bias due to an inflated type I error rate along with the fact that significance can be seen to be vary when using a number of different cut-off points for the parameter of interest, frequently resulting in an overestimated significance level on univariate analysis along with a disproportionate weighting on subsequent multivariate analysis [22–24]. Variables including preoperative CA19-9, tumour size and the lymph node ratio were, therefore, modelled as continuous covariates. CA19-9 levels were normalized for Cox modelling by logarithmic transformation (lnCA19-9).

All patients who died within 30 days of surgery were excluded from the survival analyses. Statview version 5 (SAS, Cary, N.C., USA) and Microsoft Excel (Microsoft Office 2002; Microsoft, Redmond, Wash., USA) were used to perform the various statistical functions.

Results

Relationship between Preoperative CA19-9 and Bilirubin Levels

In total, 532 consecutive patients had either resection (n = 353) or palliative bypass (n = 179) for pancreatic or periampullary tumours, of whom 297 underwent pancreatoduodenectomy. Of these, 132 had histologically confirmed pancreatic ductal adenocarcinoma. Three deaths were excluded (2%) and in 20 patients preoperative CA19-9 levels were not available, leaving 109 patients for analysis (table 1). Analysis of the excluded patient group due to missing CA19-9 data demonstrated no difference in overall median survival when compared with the study group [14.2 months (95% CI = 8.3–24.1) vs. 13.9 months (95% CI = 12.3–17.0), respectively, log-rank test, p = 0.668]. In cases where more than one CA19-9 level was recorded prior to resection, the result taken nearest to the date of surgery was used for analysis. A significant correlation between preoperative lnCA19-9 and concurrent bilirubin levels was demonstrated in 93 patients where both results were available (r = 0.265, 95% CI = 0.065–0.445, p = 0.011). 75 patients (81%) had preoperative CA19-9 and bilirubin levels recorded within 24 hours of each other, 7 (8%) within 2 days, 5 (5%) within 3 days and 6 (6%) within 3–7 days.



g. 1. Logarithmic plot of corrected p values for survival analysis
)g-rank) using various cut-off points for preoperative CA19-9
a prognostic marker.

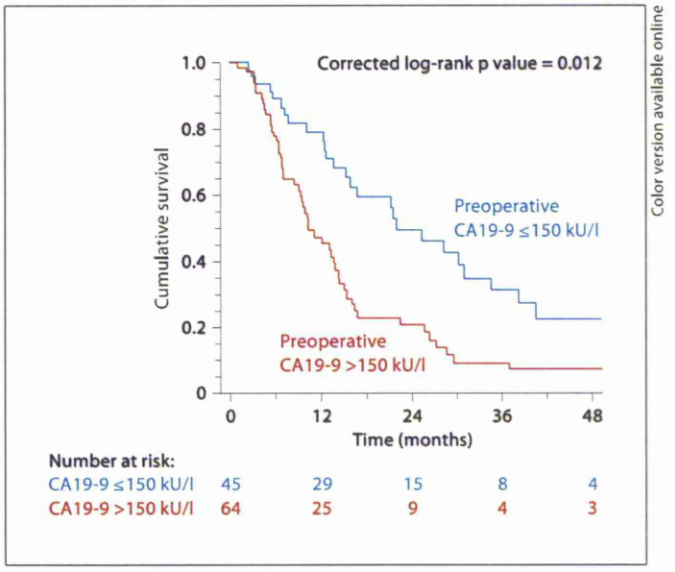


Fig. 2. Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients undergoing resection stratified by preoperative CA19-9 levels >150 kU/l.

CA19-9 and Univariate Survival

The overall median survival of the 109 patients was 3.9 months (95% CI = 12.3–17.0). There were 80 deaths nd a median follow-up time of 12.0 months (IQR = 8.3–5.1) for the 29 censored cases. Using various cut-off val-es for CA19-9, a level approximating to 150 kU/l was ound to represent the transition point at which a signif-ant survival difference was reached (fig. 1). This 'transi-on point' was recorded as the point at which the p value ecorded on the y-axis) fell below 0.01, not the lowest oint along the curve (i.e. the 'minimum p value'). A pre-perative CA19-9 of >150 kU/l was therefore selected to ratify patients for subsequent survival analyses. A cor-ected log-rank p value [22] was calculated when exclud-ng 5% of the smallest and largest values of CA19-9 as otential cut-off points using the formula $p_{cor} = -3.13p + 1.65\log_e p$).

Sixty-four patients with a CA19-9 level of >150 kU/l ad a median survival of 10.4 months (95% CI = 9.1–13.9) ompared to 22.1 months (95% CI = 15.4–34.9) for 45 pa-ents with a CA19-9 level ≤150 kU/l (log rank – p_{cor} = .012, fig. 2). Adjuvant therapy was given to 16 of 45 cas-s with a preoperative CA19-9 ≤150 kU/l, compared to 2 out of 64 with levels >150 kU/l. There were three pa-ents overall who received neo-adjuvant therapy for lo-ally advanced disease who were subsequently down-

Table 1. Distribution of preoperative CA19-9 and bilirubin parameters in resected pancreatic ductal adenocarcinoma

Patients	109
Male:female	65:44
Median age, years	66 (60–73)
Median preoperative CA19-9, kU/l	231 (66–650)
Median interval from preoperative CA19-9 to surgery, days	26 (17–37)
Cases with normal/abnormal preoperative CA19-9	
Normal (≤37 kU/l)	15 (14%)
Abnormal (>37 kU/l)	94 (86%)
Median preoperative bilirubin recorded (n = 93), μmol/l	86 (34–238)
Cases with jaundice/no jaundice when CA19-9 taken (n = 93)	
No jaundice (≤35 μmol/l)	24 (26%)
Jaundice (>35 μmol/l)	69 (74%)
Preoperative intervention for biliary drainage (n = 109)	
ERCP + stent	85 (78%)
PTC + stent/external drainage	5 (5%)
None	19 (17%)
Timing of preoperative CA19-9 (n = 81)[1]	
Before stenting	31 (38%)
After stenting	50 (62%)

Figures in parentheses represent the IQR or percentages.
[1] Cases undergoing preoperative biliary drainage where date of endoscopic retrograde cholangiopancreatography or percutaneous transhepatic cholangiography was recorded.

ble 2. Median survival times according to preoperative CA19-9 d histological subgroups

ıriable	Median overall survival, months	p (log-rank)
eoperative CA19-9		
≤150 U/ml (n = 45)	22.1 (15.4–34.9)	0.012
>150 U/ml (n = 64)	10.4 (9.1–13.9)	(corrected)
ɔdal status		
Negative (n = 21)	24.1 (14.6–63.8)	0.011
Positive (n = 88)	13.3 (10.4–15.5)	
ze[1]		
≤20 mm (n = 27)	22.5 (14.3–31.2)	0.053
>20 mm (n = 81)	13.3 (10.4–15.4)	
ımour differentiation[1]		
Well (n = 14)	22.6 (12.5–30.4)	0.066
Moderate (n = 58)	15.4 (15.4–19.7)	
Poor (n = 36)	12.6 (6.8–16.1)	
ısection margin status		
Negative (n = 29)	16.7 (12.5–30.4)	0.067
Positive (n = 80)	13.3 (10.4–15.5)	
stage		
T1/T2 (n = 21)	16.6 (12.5–37.1)	0.103
T3/T4 (n = 88)	13.7 (10.0–16.1)	

Figures in parentheses contain the IQR. Due to the small num- r of well-differentiated tumours, well- and moderately differ- tiated tumours were grouped together for analysis.
[1] Histological data incomplete for one case.

staged. Table 2 demonstrates the median survival times associated with the various histological tumour characteristics.

No significant difference was observed when comparing the median bilirubin levels (79 vs. 94 μmol/l; Mann-Whitney, p = 0.185) or the proportion of jaundiced patients (30 vs. 46%; χ^2, p = 0.290) when grouping patients according to preoperative CA19-9 levels ≤150 kU/l or >150 kU/l, respectively. The median survival of 90 patients (83%) undergoing endoscopic retrograde cholangiopancreatography or percutaneous transhepatic cholangiography for stenting or external drainage prior to resection was 14.3 months (95% CI = 12.5–21.3) compared to 13.7 months (95% CI = 6.9–17) for 19 patients (17%) who underwent no preoperative intervention for biliary drainage (log-rank – p = 0.698).

Relationship between CA19-9 and Tumour Histology

A CA19-9 level of >150 kU/l was significantly associated with tumour size, resection margin status and tumour grade, but not nodal status or tumour stage (table 3). There was a significant correlation between preoperative lnCA19-9 and tumour size [r = 0.358 (95% CI = 0.166–0.525), n = 108, p < 0.001], but not lymph node ratio [r = 0.019 (95% CI = –0.187 to 0.224), n = 107, p = 0.427]. The lymph node ratio could be calculated for 107

ble 3. Relationship between eoperative CA19-9 and histological mour characteristics (n = 109)

	Patients (stratified by preoperative CA19-9 levels)			Median CA19-9 level kU/l
	CA19-9 ≤150 kU/l	CA19-9 >150 kU/l	p (χ^2)	
Tumour size (n = 108)[1]				
≤20 mm	18	9	0.005	73 (38–258)
>20 mm	27	54		324 (116–1,009)
Nodal status (n = 109)				
Negative	11	10	0.251	128 (26–395)
Positive	34	54		246 (79–738)
T stage (n = 109)				
T1 and T2	10	11	0.512	162 (41–493)
T3 and T4	35	53		233 (71–713)
Resection margin (n = 109)				
Negative	17	12	0.027	90 (45–476)
Positive	28	52		280 (107–947)
Differentiation (n = 108)[1]				
Well/moderate	36	36	0.023	162 (51–610)
Poor	9	27		284 (152–771)

Figures in parentheses contain the IQR.
[1] Histological data incomplete for one case.

ıses. The median number of lymph nodes sampled was ' (IQR = 11–25) and the median lymph node ratio for ɔde positive cases was 0.24 (IQR = 0.16–0.33).

Postoperative CA19-9 and Survival

56 out of 94 patients with an elevated preoperative A19-9 level (>37 kU/l) had postoperative CA19-9 levels :corded within 3 months of resection at a median inter-ıl of 47 days (IQR = 34–66). The median postoperative A19-9 was 47 kU/l (IQR = 23–138). There were 25 pa-ents with a normal CA19-9 after resection with a me-ian survival of 28.4 months (95% CI = 14.6–38.5) com-ared with 10.4 months (95% CI = 9.1–14.3) for 31 patients ith an elevated postoperative CA19-9 (corrected log-ınk p = 0.012; fig. 3).

Eleven out of 12 patients with a normal preoperative A19-9 level in whom postoperative levels were record-l within 3 months of surgery had normal postoperative vels [median survival = 17.0 months (95% CI = 13.8–).7)] compared with one patient where the postopera-ve CA19-9 rose to 133 kU/l – this patient survived 7.5 ıonths. There was a significantly reduced likelihood 'isher's exact test, $p < 0.001$) of postoperative CA19-9 ɔrmalisation in cases with preoperative CA19-9 levels 150 kU/l (9 out of 36) compared with cases who had reoperative CA19-9 levels of 38–150 kU/l (16 out of ɔ)).

Multivariate Survival Analysis

Of the five variables selected from univariate analysis, only the preoperative CA19-9 (p = 0.030) and lymph node ratio (p = 0.042) maintained significance on multivariate analysis (table 4).

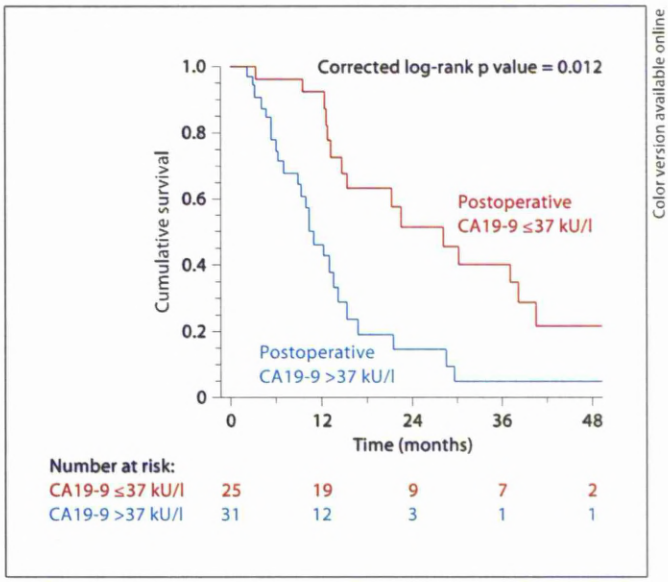


Fig. 3. Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma cases according to postoperative normalisation of CA19-9.

ıble 4. Univariate and multivariate ırvival analysis (Cox proportional ızards) for CA19-9 and histological ɔgnostic factors

Prognostic markers	Univariate analysis, p value	Multivariate analysis (n = 105)	
		Hazard ratio	p value
lnCA19-9[1] (n = 109)	0.001	1.17 (1.02–1.35)	0.030
Lymph node ratio[1] (n = 107)	0.003	3.75 (1.05–13.35)	0.042
Tumour size[1] (n = 108)	0.004	1.02 (1.00–1.04)	0.071
Tumour differentiation (n = 108)			
Well/moderate	0.066	1.24 (0.74–2.07)	0.421
Poor			
Resection margin status (n = 109)			
Negative	0.067	1.001 (0.52–1.93)	0.998
Positive			

Figures in parentheses are the 95% CI values.

[1] Modelled as continuous covariates on both univariate and multivariate analysis; hazard ratios for continuous data reflect an increase in relative risk of death with each incremental increase in the covariate value of 1 unit. Histological data was incomplete for a small number of patients, hence the final multivariate model included 105 cases.

Discussion

Several histological tumour characteristics have been ɔnsistently demonstrated to have significant prognostic alue in resected pancreatic adenocarcinoma [25–30]. fost notably, these include tumour size, nodal involveıent (including lymph node 8a status), differentiation and :section margin status. A number of molecular markers ave also been shown to be of prognostic value following :section[31–35]; however, these tumour characteristics, hether histological or biological in nature, are invariably nly amenable to assessment following surgery.

The results from the present study confirm that great- : preoperative CA19-9 levels are associated with a sigificantly reduced overall survival following resection ɔr pancreatic ductal adenocarcinoma. A clear statistical ıtionale is provided for the selection of a cut-off value of 50 kU/l in this analysis. Previous studies [14–16] that ave investigated the potential prognostic value of preop- :ative CA19-9 levels in resected pancreatic cancer di- ıotomized the number of patients into high- and low- sk groups according to a single cut-off value for CA19-9 ithout any attempt to correct for the potential bias as- ɔciated with this approach. The present study uses a :cognised method for correcting the quoted univariate ɔg-rank p values [22] and the multivariate analysis was ɔnducted utilising continuous covariates in order to oid any bias associated with categorising continuous rognostic data [22–24].

The issue of concurrent jaundice as a confounding fac- ɔr in the interpretation of CA19-9 levels for prognostic urposes does not appear to be of importance in this con- :xt. When comparing the two patient groups stratified y preoperative CA19-9 levels, there was no significant ifference in either the median bilirubin levels recorded r the overall proportion of jaundiced and non-jaundiced ıses in each group. The strength of association between reoperative CA19-9 and bilirubin levels in the present udy, while significant, only returned a relatively small oefficient with a wide confidence interval, which includ- l values of very weak correlation. These results are consstent with the findings from a previous study [8] which emonstrated that, in the context of diagnosing pancre- ic malignancy in symptomatic patients, the presence of ɔncurrent obstructive jaundice did not significantly af- :ct the sensitivity or specificity of CA19-9 when using a :andard diagnostic cut-off value of 37 kU/l.

The deleterious survival outcome observed for cases ith preoperative CA19-9 levels >150 kU/l was related to ıe association with a larger, more poorly differentiated tumour along with microscopic resection margin involvement. These findings support the notion that preoperative CA19-9 levels are not only indicative of tumour burden, but also that CA19-9 levels may act as a marker of biological 'aggressiveness'. This hypothesis is borne out by the finding that preoperative CA19-9 was a more significant variable when compared to tumour size or differentiation status alone. Given that the presence of local or distant micrometastases at the time of surgery is believed to be the most significant factor in limiting long-term survival for the majority of resected pancreatic cancer patients [36], it is a reasonable hypothesis to suppose that preoperative CA19-9 levels may also act as a marker of disseminated micrometastatic disease.

The lymph node ratio emerged as the only other significant prognostic marker on multivariate analysis when analysed as a continuous covariate. This parameter has recently been demonstrated to reflect an important prognostic index in a large series of patients undergoing resection for pancreatic ductal adenocarcinoma [17]. The lymph node ratio has also been shown to be a significant prognostic index following resection for both colorectal [37] and gastric malignancy [38], and is likely to be increasingly used in future studies as a more representative reflection of overall nodal status following resection.

Analysis of postoperative changes in CA19-9 demonstrated the expected improved survival associated with normalisation of levels within 3 months of surgery. The data also indicated that patients with a preoperative CA19-9 level >150 kU/l were significantly less likely to experience normalisation of levels postoperatively.

These observations have two important implications. Firstly, the role of prognostic molecular markers [31–35] should be properly validated in studies that also include preoperative CA19-9 levels and the lymph node ratio obtained by analysis of the tumour specimen. Secondly, stratification of patients according to both of these variables should be considered in future adjuvant trials [39, 40].

Acknowledgements

The authors are grateful to Dr. Catrin Tudor-Smith PhD (Senior Lecturer in Medical Statistics, School of Cancer Studies, University of Liverpool) for statistical advice, and to Cancer Research UK, who provided financial support for this study.

References

1 Koprowski H, Steplewski Z, Mitchell K, Herlyn M, Herlyn D, Fuhrer P, et al. Colorectal carcinoma antigens detected by hybridoma antibodies. Somat Cell Genet 1979;5:957–972.

2 Satomura Y, Sawabu N, Takemori T, Ohta H, Watanbe H, Okai T, et al: Expression of various sialyated carbohydrate antigens in malignant and non-malignant pancreatic tissues. Pancreas 1991;6:448–458.

3 Ker CG, Chen JS, Lee KT, Sheen PC, Wu CC: Assessment of serum and bile level of CA19-9 and CA125 in cholangitis and bile duct carcinoma. J Gastroenterol Hepatol 1991;6:505–508.

4 von Ritter C, Eder MI, Stieber P, Lamerz R, Jüngst D, Strigl M, et al: Biliary mucin secreted by cultured human gallbladder epithelial cells carries the epitope of CA19-9. Anticancer Res 1997;17:2931–2934.

5 Brockmann J, Emparan C, Hernandez CA, Sulkowski U, Dietl KH, Menzel J, et al: Gallbladder bile tumor marker quantification for detection of pancreato-biliary malignancies. Anticancer Res 2000;20:4941–4947.

6 Duraker N, Hot S, Polat Y, Höbek A, Gencler N, Urhan N: CEA, CA19-9 and CA125 in the differential diagnosis of benign and malignant pancreatic disease with or without jaundice. J Surg Oncol 2007;95:142–147.

7 Itzjowitz S, Kim Y: New carbohydrate tumour markers. Gastroenterology 1986;90:491–494.

8 Kim HJ, Kim MH, Myung SJ, Lim BC, Park ET, Yoo KS, et al: A new strategy for the application of CA19-9 in the differentiation of pancreato-biliary cancer: analysis using a receiver operating characteristic curve. Am J Gastroenterol 1999;94:1941–1946.

9 Connor S, Bosonnet L, Alexakis N, Raraty M, Ghaneh P, Sutton R, et al: Serum CA19-9 measurement increases the effectiveness of staging laparoscopy in patients with suspected pancreatic malignancy. Dig Surg 2005;22:80–85.

10 Mann DV, Edwards R, Ho S, Lau WY, Glazer G: Elevated tumour marker CA19-9: clinical interpretation and influence of obstructive jaundice. Eur J Surg Oncol 2000;26:474–479.

11 Sperti C, Pasquali C, Catalini S, Cappellazzo F, Bonadimani B, Behboo R, et al. CA19-9 as a prognostic index after resection for pancreatic cancer. J Surg Oncol 1993;52:137–141.

12 Montgomery RC, Hoffman JP, Riley LB, Rogatko A, Ridge JA, Eisenberg BL, et al: Prediction of recurrence and survival by post-resection CA19-9 values in patients with adenocarcinoma of the pancreas. Ann Surg Oncol 1997;4:551–556.

13 Safi F, Schlosser W, Falkenreck S, Beger HG: Prognostic value of CA19-9 serum course in pancreatic cancer. Hepatogastroenterology 1998;45:253–259.

14 Kau SY, Shyr YM, Su CH, Wu CW, Lui WY: Diagnostic and prognostic values of CA19-9 and CEA in periampullary cancers. J Am Coll Surg 1999;188:415–420.

15 Lundin J, Roberts PJ, Kuusela P, Haglund C: The prognostic value of preoperative serum levels of CA19-9 and CEA in patients with pancreatic cancer. Br J Cancer 1994;69:515–519.

16 Ferrone CR, Finkelstein DM, Thayer SP, Muzikansky A, Fernandez del-Castillo C, Warshaw AL: Perioperative CA19-9 levels can predict stage and survival in patients with pancreatic ductal adenocarcinoma. J Clin Oncol 2006;24:2897–2902.

17 Pawlik TM, Gleisner AL, Cameron JL, Winter JM, Assumpcao L, Lillemoe KD, et al: Prognostic relevance of lymph node ratio following pancreaticoduodenectomy for pancreatic cancer. Surgery 2007;141:610–618.

18 Berger AC, Watson JC, Ross EA, Hoffman JP: The metastatic/examined lymph node ratio is an important prognostic factor after pancreaticoduodenectomy for pancreatic ductal adenocarcinoma. Ann Surg 2004;70:235–240.

19 Campbell F, Bennett M, Foulis AJ: Minimum dataset for histopathological reporting of pancreatic, ampulla of vater and Bile Duct Carcinoma. Royal College of Pathologists, 2002. http://www.rcpath.org.

20 Klöppel G, Solcia E, Longnecker DS, Capella C, Sobin LH: Histological typing of tumors of the exocrine pancreas, ed 2. International Histological Classification of Tumors. Geneva, World Health Organization, 1996. http://whqlibdoc.who.int/publications/1996/3540602801_eng.pdf.

21 Sobin LH, Wittekind CH: TNM Classification of Malignant Tumours, ed 6. New York, Wiley, 2002.

22 Altman DG, Lausen B, Sauerbrei W, Schumacher H: Dangers of using optimal cutpoints in the evaluation of prognostic factors. J Nat Cancer Inst 1994;86:829–835.

23 Holländer N, Shumacher M: On the problem of using 'optimal' cutpoints in the assessment of quantitative prognostic factors. Onkologie 2001;24:194–199.

24 Holländer N, Sauerbrei W, Schumacher M: Confidence intervals for the effect of a prognostic factor after selection of an 'optimal' cutpoint. Stat Med 2004;23:1701–1713.

25 Benassi G, Mastrorilli M, Quarto G, Cappielo A, Giani U, Forestieri P, et al: Factors influencing survival after resection for ductal adenocarcinoma of the head of pancreas. J Surg Oncol 2000;73:212–218.

26 Neoptolemos JP, Stocken DD, Dunn JA, Almond J, Beger HG, Pederzoli P, et al: Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. Ann Surg 2001;234:758–768.

27 Connor S, Bosonnet L, Ghaneh P, Alexakis N, Hartley M, Campbell F, et al: Survival of patients with periampullary carcinoma is predicted by lymph node 8a but not by lymph node 16b1 status. Br J Surg 2004;91:1592–1599.

28 Bassi C, Stocken DD, Olah A, Friess H, Buckels J, Hickey H, et al: Influence of surgical resection and post-operative complications on survival following adjuvant treatment for pancreatic cancer in the ESPAC-1 randomized controlled trial. Dig Surg 2005;22:353–363.

29 Han S, Jang J, Kim S, Kim WH, Lee KU, Park YH: Analysis of long-term survivors after surgical resection for pancreatic cancer. Pancreas 2006;32:271–275.

30 Winter JM, Cameron JL, Campbell KA, Arnold MA, Chang DC, Coleman J, et al: 1,423 pancreaticoduodenectomies for pancreatic cancer: a single-institution experience. J Gastrointest Surg 2006;10:1199–1210.

31 Bloomston M, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP, et al: MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA 2007;297:1901–1908.

32 Kim J, Reber HA, Dry SM, Elashoff D, Chen SL, Umetani N, et al: Unfavourable prognosis associated with *K-ras* gene mutation in pancreatic cancer surgical margins. Gut 2006;55:1598–1605.

33 Vimalachandran D, Greenhalf W, Thompson C, Luttges J, Prime W, Campbell F, et al: High nuclear S100A6 (calcyclin) is significantly associated with poor survival in pancreatic cancer patients. Cancer Res 2005;65:3218–3225.

34 Tascilar M, Skinner HG, Rosty C, Sohn T, Wientz RE, Offerhaus GJ, et al: The SMAD 4 protein and prognosis of pancreatic ductal adenocarcinoma. Clin Cancer Res 2001;7:4115–4121.

35 Kawesha A, Ghaneh P, Andren-Sandberg A, Ograed D, Skar R, Dawiskiba S, et al: *K-ras* oncogene subtype mutations are associated with survival but not expression of p53, p16(INK4A), p21(WAF-1), cyclin D1, erbB-2 and erbB-3 in resected pancreatic ductal adenocarcinoma. Int J Cancer 2000;89:469–474.

36 Bogoevski D, Yekebas EF, Schurr P, Kaifi JT, Kutup A, Erbersdobler A, et al: Mode of spread in the early phase of lymphatic metasis in pancreatic ductal adenocarcinoma: prognostic significance of nodal microinvolvement. Ann Surg 2004;240:993–1000.

37 Berger AC, Sigurdson ER, Le Voyer TE, Hanlon A, Mayer RJ, Macdonald JS, et al: Colon cancer survival is associated with decreasing ratio of metastatic to examined lymph nodes. J Clin Oncol 2005;23:8706–8712.

38 Inoue K, Nakane Y, Iiyama H, Sato M, Kanbara T, Nakai K, et al: The superiority of ratio-based lymph node staging in gastric carcinoma. Ann Surg Oncol 2002;9:27–34.

39 Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, et al: A randomized trial of chemotherapy after resection of pancreatic cancer. N Engl J Med 2004;350:1200–1210.

40 Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, et al: Adjuvant chemotherapy with gemcitabine vs. observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 2007;297:267–277.

The platelet-lymphocyte ratio improves the predictive value of serum CA19-9 levels in determining patient selection for staging laparoscopy in suspected periampullary cancer

Richard A. Smith, MRCS,[a] Lorraine Bosonnet, PGDip,[a] Paula Ghaneh, FRCS,[a] Robert Sutton, FRCS,[a] Johnathan Evans, FRCR,[b] Priya Healey, FRCR,[b] Connall Garvey, FRCR,[b] Mark Hughes, FRCR,[b] Michael Raraty, FRCS,[a] Fiona Campbell, FRCPath,[c] *and* John P. Neoptolemos, FRCS,[a] *Liverpool, UK*

Background. *The objective of this study was to identify whether the preoperative platelet-lymphocyte (P/L) ratio might improve the predictive value of CA19-9 levels in stratifying a patient group with suspected periampullary malignancy who do not require staging laparoscopy.*
Methods. *Patients with suspected periampullary cancer were identified from a prospectively maintained 10-year database. Only patients with resectable disease who underwent staging laparoscopy and subsequent laparotomy were included. Low-risk groups were stratified using a CA19-9 cutoff value of ≤ 150 kU/l (or ≤ 300 kU/l in patients with a concurrent bilirubin concentration > 35 μmol/l) and a P/L ratio value of ≤ 150.*
Results. *From 263 patients, preoperative CA19-9 levels and P/L ratios were available in 216 and 225 patients, respectively. The positive and negative predictive values for resectability, sensitivity, and specificity for CA19-9 levels ≤ 150kU/l were 83%, 36%, 51%, and 73%, respectively. For P/L ratios ≤ 150, these levels were 81%, 38%, 51%, and 72%, respectively. When combining the requirement for both CA19-9 levels and P/L ratios to be ≤ 150 (n = 38 out of 183), both positive predictive value (95%) and specificity (96%) were improved (Fisher exact test, P = .065 and P < .001, respectively); 21% of laparoscopies were avoidable when using these criteria. Increasing T stage (P = .005), vascular invasion (P < .001), perineural invasion (P = .008), and resection margin involvement (P < .001) were all associated with greater preoperative P/L ratios in resected periampullary adenocarcinoma (n = 204).*
Conclusions. *The preoperative P/L ratio reflects an index of tumor invasiveness and merits prospective evaluation as an adjunct to CA19-9 in determining the requirement for laparoscopic staging in patients with potentially resectable periampullary malignancy. (Surgery 2008;143:658-66.)*

From the Division of Surgery and Oncology, School of Cancer Studies, University of Liverpool[a]; and Departments of Radiology[b]; and Pathology[c], Royal Liverpool University Hospital, UK

OPERATIVE RESECTION remains the only potentially curative modality for periampullary adenocarcinoma.[1] Despite the availability of modern multidetector helical computed tomography (CT), a substantial proportion of patients with periampullary cancer and radiologically resectable disease will exhibit occult metastases or locally advanced disease at laparotomy, thereby precluding the option of resection.[2-4] Preoperative staging laparoscopy using laparoscopic ultrasonography has been investigated as a supplementary modality in this setting to minimize the number of laparotomies conducted for unresectable disease.[5-7]

CA19-9 is a Lewis blood group glycolipid antigen that is used widely as a tumor marker in the preliminary diagnosis of suspected periampullary cancer, but it is known to lack specificity, because levels may be increased in the presence of pancreatobiliary conditions resulting in cholestasis.[8,9] CA19-9 has been used as an additional diagnostic tool to select a patient group with tumors that appear resectable on CT, have a low risk of occult

Supported by Cancer Research UK.
Accepted for publication December 20, 2007.
Reprint requests: Richard Smith, MRCS, Division of Surgery and Oncology, School of Cancer Studies, Royal Liverpool University Hospital, 5th Floor UCD Building, Daulby St., Liverpool, L69 3GA, UK. E-mail: rsmith3@liverpool.ac.uk.
0039-6060/$ - see front matter

doi:10.1016/j.surg.2007.12.014

metastases and locally advanced disease, and who do not require laparoscopic staging.[10-13] Use of a preoperative serum CA19-9 cutoff value of ≤ 150 kU/l (or ≤ 300 kU/l in patients with concurrent serum bilirubin concentrations > 35 μmol/l) are associated with a low incidence (5%) of advanced disease on subsequent laparoscopic assessment.[10]

Previous studies have suggested that preoperative indices of systemic inflammation might provide prognostic information in resected pancreatic cancer with increased levels of C-reactive protein,[14] thrombocytosis,[15,16] and lymphocytopenia,[17,18] all having been reported to be associated with poorer survival outcomes in patients with pancreatic cancer undergoing resection. The potential value of preoperative markers of systemic inflammation in determining resectability has not been investigated previously. The objective of this study was to identify whether the preoperative platelet-lymphocyte ratio, a proposed index of systemic inflammation, might be a clinically useful adjunct to CA19-9 levels in selecting a patient group with suspected periampullary malignancy at low risk of advanced disease at laparotomy. An additional objective of this study was to define the relationship between the platelet-lymphocyte ratio and tumor histology in a group of patients undergoing resection for periampullary adenocarcinoma.

MATERIAL AND METHODS

Details of all referrals between January 1997 and September 2006 with suspected pancreatic and periampullary malignancy were collected prospectively and maintained in a database. Patients undergoing contrast-enhanced computed tomography (CT) were identified to select patients with radiologically resectable disease. Decision-making regarding tumor resectability was undertaken during a weekly multidisciplinary team meeting. The principal CT criteria used to determine resectability were based on the presence of intra- or extra-abdominal metastatic disease and on vascular encasement or tumor involvement of the superior mesenteric-portal vein over > 50% circumference and/or > 2 cm length. Patients with equivocal CT features for resectability (ie, patients with radiologic features approximating the threshold values outlined above) who went on to undergo further staging and subsequent laparotomy were also included in the analysis.

Only patients who underwent both staging laparoscopy and attempted resection were included in the analysis. This approach was used to identify retrospectively what proportion of staging laparoscopies conducted during the study period were potentially avoidable in the patient group undergoing exploration. Laparoscopic staging included inspection of the peritoneal cavity along with intraoperative ultrasonographic assessment of the liver parenchyma and tumor relationships with local vasculature. Contraindications to laparoscopic staging included the presence of comorbid disease that would preclude consideration for further intervention, gastric outlet obstruction requiring operative bypass, or multiple previous intra-abdominal operations. Patients with proven metastatic disease from intraoperative biopsy at laparoscopy were excluded from a laparotomy. Patients with equivocal laparoscopic features of resectability who went on to undergo laparotomy and attempted resection were classified as having potentially resectable disease for the purposes of the study. Laparoscopy and laparoscopic ultrasonography were in routine use at our institution for the entire duration of the study period.

The operative criteria for unresectable disease at laparotomy were based on the finding of any hepatic or peritoneal metastases proven on frozen section, vascular encasement, or tumor involvement of the portal/superior mesenteric vein over > 2 cm precluding the option of local resection. Venous resection was performed in 6% of all pancreatoduodenectomies conducted for pancreatic and periampullary malignancy during the study period. There were no significant differences in practice between surgeons regarding the operative criteria used to determine resectability at laparotomy.

Preoperative serum CA19-9 levels, concurrent serum bilirubin concentrations, and full blood count estimations were obtained. Where more than one result was recorded, the result taken nearest to the date of surgery was used for analysis. A CA19-9 cutoff value of ≤ 150 kU/l (or ≤ 300 kU/l in patients with concurrent bilirubin concentrations > 35 μmol/l) was used to define a low-risk group for unresectable disease. This value was selected on the basis of the previously published literature.[8,10,12] The platelet-lymphocyte ratio was calculated for all patients in whom a preoperative full blood count along with differential white cell count was recorded. Various cutoff values for the platelet-lymphocyte ratio were used to determine the optimum point at which the positive predictive value for resectability was maximized. A platelet-lymphocyte ratio of ≤ 150 was found to represent this point.

All patients with suspected periampullary cancer (including those with subsequently proven benign disease) were included in the analysis as the exact

Table I. Frequency of tumor type in patients with resectable disease according to computed tomography assessment.

Histology	
Pancreatic ductal adenocarcinoma	119
Ampullary adenocarcinoma	48
Cholangiocarcinoma	34
Other malignancy	24
Metastatic adenocarcinoma (unconfirmed primary)	25
Presumed peripancreatic malignancy	4
Benign tumors	9
Total	263

origin and histologic nature of the primary is commonly unknown at the time of decision-making regarding operative intervention. Furthermore, the exact tumor origin is not established frequently in patients with locally advanced or metastatic disease identified at laparotomy. Therefore, this overall patient group reflects a more representative sample within which to study the predictive value of CA19-9 and platelet-lymphocyte ratios in a clinically meaningful setting.

A further group of patients was identified to analyze the relationship between the preoperative platelet-lymphocyte ratio and the histologic features of resected periampullary tumors. Patients undergoing pancreatoduodenectomy for histologically confirmed periampullary adenocarcinoma during the same time period (with or without laparoscopic staging) in whom a preoperative full blood count result was available were recorded. Histopathology reporting was undertaken using the Royal College of Pathologists Guidelines[19] according to the 6th edition of the International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification[20] and the 5th edition[21] prior to 2002.

STATISTICAL ANALYSIS

Continuous data were described using the median and interquartile range (IQR) with 2-tailed Mann-Whitney *U* testing or Kruskal-Wallis testing for comparative analysis. Categorical data were analyzed using chi-squared (χ^2) or Fisher exact. Receiver operating characteristic (ROC) curves were used to analyse whether serum CA19-9 levels or platelet-lymphocyte ratios conferred superior predictive information with regard to tumor resectability. Adjusted CA19-9 levels were calculated for these analyses by dividing the CA19-9 by 2 in patients with concurrent bilirubin concentrations > 35 μmol/l. The positive predictive value, negative predictive value, sensitivity, and specificity for resectability were calculated for CA19-9 levels ≤ 150 kU/l and platelet-lymphocyte ratios ≤ 150 both individually and in combination. The positive predictive value for tumor resectability reflects the likelihood of predicting correctly a resectable tumor at laparotomy in the patient group analyzed.

RESULTS

A total of 1056 patients were recorded in the database during the study period; 675 patients were identified with CT resectable or borderline resectable pancreatic or periampullary tumors of whom 336 underwent staging laparoscopy. A total of 263 patients from this group went on to laparotomy with a resection rate of 72% (190/263). The median interval between laparoscopy and operation was 14 days (IQR, 7 to 28 days). The histologic diagnoses recorded in this group are shown in Table I. A total of 149 patients were male (57%), and the median age was 65 years (IQR, 58 to 71 years).

A preoperative full blood count with differential white cell count was available in 225 patients; 188 (84%) of these patients had a full blood count recorded within 2 days of operation, 22 (10%) within 2 to 7 days, and 15 (6%) within more than 7 days prior to operation, and 216 patients had preoperative serum CA19-9 levels recorded. The median interval from the preoperative CA19-9 to date of operation was 26 days (IQR, 15 to 39 days). Concurrent serum bilirubin concentrations were available in 142 of these patients, 94 (66%) of whom had bilirubin concentrations of > 35 μmol/l. Where no concurrent serum bilirubin concentration was available, the unadjusted CA19-9 was used for analysis.

The median adjusted CA19-9 levels and platelet-lymphocyte ratios recorded for patients with resectable disease compared with locally advanced and metastatic disease at laparotomy are shown in Table II. The ROC curves for CA19-9 and platelet-lymphocyte ratios in predicting tumor resectability are shown in Fig 1. The area under the curve (AUC) recorded for the 2 prognostic indices was very similar: AUC = 0.67 (95% confidence interval [CI], 0.58-0.76) for CA19-9 and AUC = 0.68 (95% CI, 0.60-0.77) for platelet-lymphocyte ratio.

The predictive values of CA19-9 levels ≤150 kU/l in determining tumor resectability are shown in Table III. A positive predictive value, negative predictive value, sensitivity, and specificity of 83%, 36%, 51%, and 73% were recorded respectively (n = 216). The predictive values for platelet-lymphocyte

Table II. Median platelet-lymphocyte ratio and adjusted CA19-9 levels recorded in resected and unresected periampullary tumors

	Preoperative CA19-9 levels (kU/l)			*Preoperative P/L ratio*		
	n	*Median CA19-9 (IQR)*	P	*n*	*Median P/L ratio (IQR)*	P
Resected tumor	157	144 (27-569)	.001	158	147 (113-208)	<.001
Locally advanced	36	413 (106-4546)		43	202 (146-265)	
Metastatic	19	1350 (232-2554)		22	176 (139-363)	

P/L, Platelet-lymphocyte; *IQR*, interquartile range.
Quoted *P* values for Kruskal-Wallis test. CA19-9 values are adjusted according to the presence of concurrent jaundice at the time of CA19-9 estimation. The reason for unresectability was not recorded in 4 patients in whom preoperative CA19-9 levels were available and 2 patients in whom platelet-lymphocyte ratios were available.

ratios ≤ 150 were comparable broadly with values of 81%, 38%, 51%, and 72%, respectively (n = 225). The predictive values of using the combined requirement for both CA19-9 and platelet-lymphocyte ratio to be ≤ 150 were 95%, 35%, 28%, and 96%, respectively (n = 183). These combined criteria resulted in an improved specificity over using CA19-9 in isolation (Fisher exact test, $P < .001$) and a borderline improvement in the positive predictive value for resectability (Fisher exact test, $P = .065$). If using both CA19-9 and platelet-lymphocyte ratio to guide decision-making regarding the requirement for preoperative laparoscopic staging, 21% (38/183) of laparoscopies would have been avoided with a false-positive rate for resectability at laparotomy of only 5% (2/38).

The median platelet-lymphocyte ratios associated with the various histologic features of 204 resected periampullary tumors for which a preoperative full blood count result was available are shown in Table IV. This group comprised 113 patients with pancreatic ductal adenocarcinoma, 53 with ampullary adenocarcinoma, and 38 with intrapancreatic bile duct adenocarcinoma. Increasing platelet-lymphocyte ratios were associated strongly with T-stage ($P = .005$), perineural invasion ($P = .008$), vascular invasion ($P < .001$), and involvement of the resection margin ($P < .001$). Figure 2 illustrates the association between increasing median platelet-lymphocyte ratios and increasing T-stage in this group.

A separate analysis was conducted in this patient group to identify whether preoperative biliary stenting had any impact on the platelet-lymphocyte ratio recorded prior to operation. Fully 174 patients (85%) underwent some form of biliary drainage preoperatively (165 at endoscopic retrograde cholangiopancreatography and 9 at percutaneous transhepatic cholangiography). The median interval from stenting to surgery was 34 days (IQR, 21 to 49 days). No difference in the median preoperative platelet-lymphocyte ratio was demonstrated when comparing those patients who underwent biliary drainage (median platelet-lymphocyte ratio, 157 [IQR, 113 to 219]) with those who did not (median platelet-lymphocyte ratio, 156 [IQR, 120 to 327]) with the Mann-Whitney *U* test ($P = .352$).

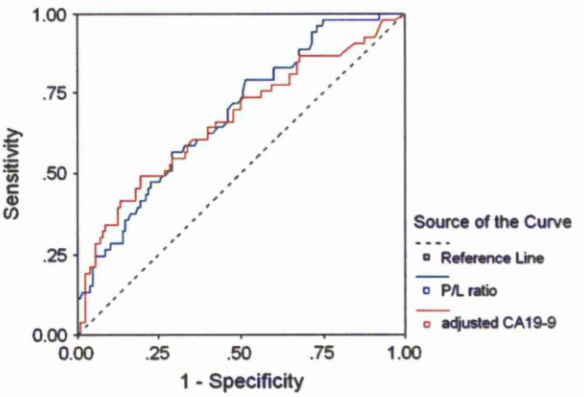


Fig 1. Receiver operating characteristic (ROC) curves to compare the predictive values of preoperative CA19-9 and platelet-lymphocyte (P/L) ratio in determining disease resectability at laparotomy.

DISCUSSION

Laparoscopic staging has been demonstrated to influence decision-making regarding operative intervention in approximately 15% of patients with radiologically resectable periampullary malignancy.[5,22] Use of staging laparoscopy can minimize potentially unnecessary operative intervention in patients with locally advanced and metastatic disease missed by CT imaging and facilitate earlier administration of the most appropriate palliative therapy.[23-26] Although endoscopic ultrasonography represents a potential alternative staging modality to image tumor relationships with local vasculature along with regional adenopathy,[27] the ability to inspect visually the peritoneal cavity and liver surface to exclude small metastatic deposits

Table III. Contingency table for predictive values of preoperative CA19-9 and platelet-lymphocyte ratio in determining periampullary tumor resectability at laparotomy

		Resectable cases	*Unresectable cases*	*Predictive values (%)*
Preoperative CA19-9 levels*	≤ 150 kU/l (n = 96)	80	16	PPV (83) NPV (36)
	> 150 kU/l (n = 120)	77	43	Sensitivity (51) Specificity (73)
Preoperative P/L ratio	≤ 150 (n = 100)	81	19	PPV (81) NPV (38)
	> 150 (n = 125)	77	48	Sensitivity (51) Specificity (72)
Combined CA19-9* and P/L ratio	≤ 150 for both (n = 38)	36	2	PPV (95) NPV (35)
	> 150 for either (n = 145)	94	51	Sensitivity (28) Specificity (96)

PPV, Positive predictive value; *NPV*, negative predictive value; *P/L*, platelet-lymphocyte.
*CA19-9 cutoff value of ≤ 300 kU/l used in jaundiced patients (ie, where concurrent bilirubin > 35 μmol/l).

represents the principal advantage of laparoscopy over other staging modalities. Staging laparoscopy has also been demonstrated to be as useful in influencing operative decision-making for periampullary tumors of nonpancreatic origin.[28]

Increased serum levels of CA19-9 represent a reliable marker of metastatic disease,[12] but CA19-9 levels are believed to be relatively less effective at identifying locally advanced disease.[10] Low preoperative CA19-9 levels have been investigated previously as a potential means of reliably identifying patients with resectable periampullary tumors at laparotomy, thereby avoiding the requirement for supplementary staging in all patients.[10-13] This approach presents the opportunity to make more judicious use of staging laparoscopy, which is particularly relevant in centers where laparoscopic staging is conducted routinely on separate theatre sessions prior to laparotomy. Previous single-center studies have reported favorable results associated with palliative gastrojejunostomy for gastric outlet obstruction due to inoperable malignancy in small patient series.[29,30] No studies have been conducted to date, however, to suggest that routine laparoscopic palliative duodenal bypass represents an optimal management approach to prevent gastric outlet obstruction in patients for whom inoperable periampullary malignancy is identified prior to exploration.

Invasive cancer causes tissue damage adjacent to the tumor, which results in both a local and systemic chronic inflammatory response. Pancreatic cancer in particular is characterized by a marked, desmoplastic stromal reaction on microscopic examination that reflects an intense inflammatory and fibrotic host reaction to tumor.[31] Inflammation results in release of both proinflammatory and inhibitory immunologic mediators. Interleukin (IL)-10 and transforming growth factor-β represent the most important inhibitory cytokines that can result in depressed lymphocyte function and reduced circulating lymphocyte counts.[32] Pancreatic cancer cells have also been demonstrated to secrete directly both IL-10 and TGF-β2, thereby providing a mechanism via which the tumor can evade immune surveillance.[33] Lymphocytopenia is associated with other gastrointestinal malignancies, including colorectal and gastric cancer.[34]

Megakaryocyte proliferation is promoted by a number of proinflammatory cytokines including IL-1, IL-3, and IL-6,[35,36] resulting in thrombocytosis. Increased platelet counts are also associated with several other malignancies including colorectal,[37] gastric,[38] and esophageal cancer.[39] Thrombocytosis and lymphocytopenia both correlate with the degree of host systemic inflammation, and the platelet-lymphocyte ratio reflects a novel marker incorporating both hematologic indices. No previous studies to our knowledge have investigated this parameter in any clinical setting.

The results of the present study suggest that the preoperative platelet-lymphocyte ratio correlates with features of local tumor invasiveness in patients with resected periampullary adenocarcinoma. Increasing T-stage, vascular invasion, and involvement of the resection margin were associated with a trend toward greater median preoperative platelet-lymphocyte ratios in each of the three periampullary tumor groups. Although tumors larger than 2 cm in diameter also resulted in a greater median platelet-lymphocyte ratio for ampullary and distal bile duct cancers, only ampullary cancers exhibited an association between nodal involvement and platelet-lymphocyte ratio,

Table IV. Median preoperative platelet-lymphocyte ratios according to histologic tumor characteristics for 204 resected periampullary adenocarcinomas

	Pancreas			*Ampulla*			*Distal Bile Duct*			*Overall*		
Variable	*n*	*Median P/L ratio*	P	*n*	*Median P/L ratio*	P	*n*	*Median P/L ratio*	P	*n*	*Median P/L ratio*	P
T stage												
T1	6	105 (90 to 143)	.061	9	129 (94 to 135)	.134	0	-	.070	15	118 (90 to 140)	.005
T2	11	155 (123 to 216)		12	159 (117 to 170)		3	69 (67 to 115)		26	156 (100 to 180)	
T3	93	173 (121 to 257)		27	153 (112 to 251)		34*	157 (117 to 218)		154	163 (118 to 248)	
T4	2	354 (284 to 424)		5	190 (142 to 245)		1*			8	202 (141 to 306)	
Nodal status												
Negative	21	180 (140 to 257)	.560	24	123 (86 to 149)	.002	9	162 (122 to 212)	.571	54	144 (107 to 198)	.173
Positive	92	159 (116 to 230)		29	171 (138 to 246)		29	151 (112 to 209)		150	159 (116 to 230)	
Size												
≤20mm	28	153 (110 to 199)	.351	28	129 (105 to 169)	.023	12	111 (94 to 165)	.044	68	134 (106 to 183)	.003
>20mm	83	168 (121 to 252)		24	168 (139 to 247)		24	161 (126 to 241)		131	163 (125 to 244)	
Resection margin status												
Negative	32	150 (110 to 193)	.043	31	136 (95 to 163)	.032	8	125 (92 to 167)	.224	71	139 (106 to 184)	<.001
Positive	80	187 (125 to 292)		22	175 (136 to 232)		30	158 (121 to 221)		132	172 (124 to 247)	
Perineural invasion												
Negative	6	114 (106 to 165)	.185	22	129 (100 to 187)	.086	4	107 (92 to 130)	.107	32	122 (99 to 183)	.008
Positive	100	161 (117 to 251)		16	169 (140 to 248)		33	159 (122 to 224)		149	162 (124 to 244)	
Vascular invasion												
Negative	28	140 (94 to 187)	.015	21	129 (83 to 165)	.022	13	124 (100 to 173)	.076	62	139 (106 to 184)	<.001
Positive	70	174 (127 to 266)		24	165 (134 to 245)		22	163 (129 to 248)		116	172 (124 to 247)	

P values for 2-sided Mann-Whitney *U* test; Kruskal-Wallis test conducted for T-stage. Histologic data was incomplete for a small number of patients.
*T3 and T4 tumors were analyzed collectively (Mann-Whitney U test).

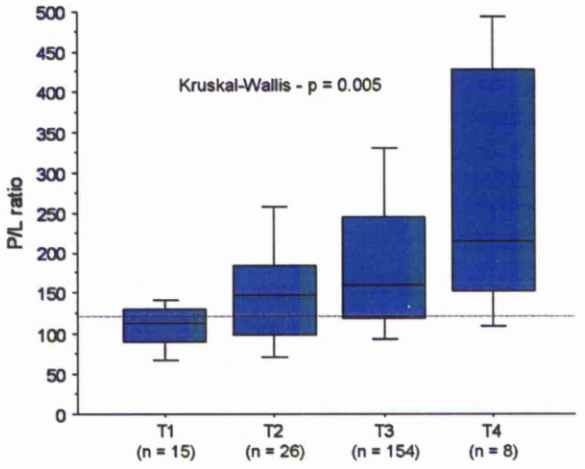


Fig 2. Box plots to illustrate relationship between median preoperative platelet-lymphocyte (P/L) ratios and T-stage in 204 resected periampullary adenocarcinomas. *Dotted line* reflects the "average" expected platelet-lymphocyte ratio for the normal population (ie, dividing the midpoint of the normal reference range for the platelet count [150-400 $x10^9$/l] by the midpoint for the normal reference range of the lymphocyte count [1.0-3.5 $x10^9$/l] – 275 ÷ 2.25 = 122).

suggesting that a more marked host systemic immune response is elicited according to the extent of local tumor invasion rather than metastatic spread. This observation is also supported by the fact that a greater median platelet-lymphocyte ratio was observed in patients with locally advanced periampullary tumors when compared with those with metastatic disease (Table II). This observation contrasts with the results for CA19-9, which demonstrated greater median CA19-9 values in patients with metastatic disease at laparotomy, a finding consistent with previous studies.[12]

Analysis of the ROC curves indicates that the overall predictive value of the preoperative platelet-lymphocyte ratio is comparable with that seen for adjusted CA19-9 levels. The contingency tables for CA19-9 and platelet-lymphocyte ratio also resulted in a broadly comparable positive predictive value and specificity for both. The combined use of CA19-9 levels ≤ 150 kU/l (or ≤ 300 kU/l in jaundiced patients) and a platelet-lymphocyte ratio ≤ 150 resulted, however, in a significant improvement in the ability to identify a low-risk group for unresectable disease at laparotomy with a positive predictive value for resectability of 95% and a specificity of 96%. Use of these combined criteria for selective use of staging laparoscopy would have resulted in 38 of 183 laparoscopies (21%) being avoided, with a false-positive rate of only 5% (ie, only 5% of patients going straight to laparotomy with unresectable disease). The poor negative predictive value and sensitivity for CA19-9 and platelet-lymphocyte ratios indicate that neither parameter can predict reliably *unresectable* periampullary tumors and, as such, investigating the predictive values of these parameters in the patient group with convincing evidence of advanced disease diagnosed at CT or laparoscopy would not result in any information that would alter decision-making regarding operative exploration.

It has been estimated previously that less than 5% of the overall population lack the Lewis antigen glycosyl transferase enzyme required to synthesize CA19-9.[40] This represents a potential confounding factor in interpreting the predictive values associated with CA19-9; however, only 5 of 216 patients (~2%) in the present study for whom a preoperative CA19-9 was recorded had unrecordable CA19-9 levels (< 2 kU/l). These patients were included in the analysis to avoid potential bias, but this issue is unlikely to have any relevant impact on the validity of the CA19-9 predictive values recorded in this study.

Preoperative biliary stenting represents an additional, potential confounding factor in interpreting the predictive values of the platelet-lymphocyte ratio. Instrumentation of the bile duct (whether endoscopic or percutaneous) is associated with a risk of biliary sepsis prior to operation, which may represent a potential cause for elevated preoperative inflammatory markers. A previous study,[41] however, demonstrated that the incidence of post-stenting cholangitis prior to pancreatoduodenectomy is only 7%. Furthermore, although the majority of patients with resected periampullary cancer underwent preoperative biliary drainage procedures in the present study, no difference in the median platelet-lymphocyte ratio was recorded when comparing patients who did or did not undergo biliary stenting. Given this finding, preoperative intervention for biliary obstruction was not deemed to be a significant confounding factor in interpreting the results.

In summary, our study suggests an association between preoperative inflammation and periampullary cancer resectability. The preoperative platelet-lymphocyte ratio was associated with both macroscopic and microscopic features of periampullary tumor invasiveness and appears to be a more effective marker of locally advanced disease than CA19-9. Use of both CA19-9 and platelet-lymphocyte ratio in risk-stratifying patients with suspected periampullary malignancy for staging laparoscopy resulted in a significant improvement in the ability

to identify those patients in whom supplementary staging can be avoided safely. The results of this study suggest that the preoperative platelet-lymphocyte ratio merits prospective evaluation alongside CA19-9 in this setting.

REFERENCES

1. Alexakis N, Halloran C, Raraty M, et al. Current standards of surgery for pancreatic cancer. Br J Surg 2004;91:1410-27.
2. Bipat S, Phoa SS, van Delden OM, et al. Ultrasonography, computed tomography and magnetic resonance imaging for diagnosis and determining resectability of pancreatic adenocarcinoma: a meta-analysis. J Comput Assist Tomogr 2005;29:438-45.
3. House MG, Yeo CJ, Cameron JL, et al. Predicting resectability of periampullary cancer with three-dimensional computed tomography. J Gastrointest Surg 2004;8:280-8.
4. Midwinter MJ, Beveridge CJ, Wilsdon JB, et al. Correlation between spiral computed tomography, endoscopic ultrasonography and findings at operation in pancreatic and ampullary tumours. Br J Surg 1999;86:189-93.
5. Doran HE, Bosonnet L, Connor S, et al. Laparoscopy and laparoscopic ultrasound in the evaluation of pancreatic and periampullary tumours. Dig Surg 2004;21:305-13.
6. Vollmer CM, Drebin JA, Middleton WD, et al. Utility of staging laparoscopy in subsets of peripancreatic and biliary malignancies. Ann Surg 2002;235:1-7.
7. Taylor AM, Roberts SA, Manson JM. Experience with laparoscopic ultrasonography for defining tumour resectability in carcinoma of the pancreatic head and peri-ampullary region. Br J Surg 2001;88:1077-83.
8. Kim HJ, Kim MH, Myung SJ, et al. A new strategy for the application of CA19-9 in the differentiation of pancreatobiliary cancer: analysis using a receiver operating characteristic curve. Am J Gastroenterol 1999;94:1941-6.
9. Brockmann J, Emparan C, Hernandez CA, et al. Gallbladder bile tumor marker quantification for detection of pancreato-biliary malignancies. Anticancer Res 2000;20: 4941-7.
10. Connor S, Bosonnet L, Alexakis N, et al. Serum CA19-9 measurement increases the effectiveness of staging laparoscopy in patients with suspected pancreatic malignancy. Dig Surg 2005;22:80-5.
11. Karachristos A, Scarmeas N, Hoffman JP. CA19-9 levels predict results of staging laparoscopy in pancreatic cancer. J Gastrointest Surg 2005;9:1286-92.
12. Schlieman MG, Ho HS, Bold RJ. Utility of tumour markers in determining resectability of pancreatic cancer. Arch Surg 2003;138:951-6.
13. Halloran CM, Ghaneh P, Connor S, et al. CA19–9 accurately selects patients for laparoscopic assessment to determine resectability of pancreatic malignancy. Br J Surg 2007;95:453-59.
14. Jamieson NB, Glen P, McMillan DC, et al. Systemic inflammatory response predicts outcome in patients undergoing resection for ductal adenocarcinoma of the head of pancreas. Br J Cancer 2005;92:21-3.
15. Suzuki K, Aiura K, Kitagou M, et al. Platelet counts closely correlate with the disease-free survival interval of pancreatic cancer patients. Hepatogastroenterology 2004;51:847-53.
16. Brown KM, Domin C, Aranha GV, et al. Increased preoperative platelet count is associated with decreased survival after resection for adenocarcinoma of the pancreas. Am J Surg 2005;189:278-82.
17. Yamaguchi K, Noshiro H, Shimizu S, et al. Long-term and short-term survivors after pancreatectomy for pancreatic cancer. Int Surg 2000;85:71-6.
18. Fogar P, Sperti C, Basso D, et al. Decreased total lymphocyte counts in pancreatic cancer: an index of adverse outcome. Pancreas 2006;32:22-8.
19. Campbell F, Bennett M, Foulis AJ. Minimum dataset for histopathological reporting of pancreatic, ampulla of Vater and bile duct carcinoma. The Royal College of Pathologists Web site 2002 Available http://www.rcpath.org/index.asp?pageId=1163. Accessed 02/01/2008.
20. Sobin LH, Wittekind Ch, editors. TNM classification of malignant tumours. 6th ed. New York: Wiley-Liss; 2002.
21. Sobin LH, Wittekind Ch, editors. TNM classification of malignant tumours. 5th ed. New York: Wiley-Liss; 1997.
22. Minnard EA, Conlon KC, Hoos A, et al. Laparoscopic ultrasound enhances standard laparoscopy in the staging of pancreatic cancer. Ann Surg 1998;228:182-7.
23. Nieveen van Dijkum EJ, Romijn MG, Terwee CB, et al. Laparoscopic staging and subsequent palliation in patients with peripancreatic carcinoma. Ann Surg 2003;237:66-73.
24. Doucas H, Sutton CD, Zimmerman A, et al. Assessment of pancreatic malignancy with laparoscopy and intraoperative ultrasound. Surg Endosc 2007;21:1147-52.
25. Ellsmere J, Moltele K, Sahari D, et al. Does multidetector-row CT eliminate the role of diagnostic laparoscopy in assessing the resectability of pancreatic head adenocarcinoma? Surg Endosc 2005;19:369-73.
26. Thomson BN, Parks RW, Redhead DN, et al. Refining the role of laparoscopy and laparoscopic ultrasound in the staging of presumed pancreatic head and ampullary tumours. Br J Cancer 2006;94:213-7.
27. Dewitt J, Devereaux BM, Lehman GA, et al. Comparison of endoscopic ultrasound and computed tomography for the preoperative evaluation of pancreatic cancer: a systematic review. Clin Gastroenterol Hepatol 2006;4:717-25.
28. Brooks AD, Mallis MJ, Brennan MF, et al. The value of laparoscopy in the management of ampullary, duodenal and distal bile duct tumours. J Gastrointest Surg 2002;6:139-46.
29. Suzuki O, Shichinohe T, Yano T, et al. Laparoscopic modified Devine exclusion gastrojejunostomy as a palliative surgery to relieve malignant pyloroduodenal obstruction by unresectable cancer. Am J Surg 2007;194:416-8.
30. Kazanjian KK, Reber HA, Hines OJ. Laparoscopic gastrojejunostomy for gastric outlet obstruction in pancreatic cancer. Am Surg 2004;70:910-3.
31. Kloppel G, Solcia E, Longnecker DS, et al. Histological typing of tumours of the exocrine pancreas, 2nd ed. WHO international histological classification of tumours. Berlin: Springer-Verlag; 1996.
32. Salazar-Onfray F, Lopez MN, Mendoza-Naranjo A. Paradoxical effects of cytokines in tumor immune surveillance and tumor escape. Cytokine Growth Factor Rev 2007;18:171-82.
33. Bellone G, Turletti A, Artusio E, et al. Tumor-associated transforming growth factor-beta and interleukin-10 contribute to a systemic Th2 immune phenotype in pancreatic carcinoma patients. Am J Pathol 1999;155:537-47.
34. Romano F, Uggeri F, Crippa S, et al. Immunodeficiency in different histotypes of radically operable gastrointestinal cancers. J Exp Clin Cancer Res 2004;23:195-200.
35. Klinger MH, Jelkmann W. Role of blood platelets in infection and inflammation. J Interferon Cytokine Res 2002;22: 913-22.
36. Alexandrakis MG, Passam FH, Moschandrea IA, et al. Levels of serum cytokines and acute phase proteins in patients

with essential and cancer-related thrombocytosis. Am J Clin Oncol 2003;26:135-40.
37. Monreal M, Fernandez-Llamazares J, Pinol M, et al. Platelet count and survival in colorectal cancer—a preliminary study. Thromb Haemost 1998;79:916-8.
38. Ikeda M, Furukawa H, Imamura H, et al. Poor prognosis associated with thrombocytosis in patients with gastric cancer. Ann Surg Oncol 2002;9:287-91.
39. Shimada H, Oohira G, Okazumi S, et al. Thrombocytosis associated with poor prognosis in patients with oesophageal carcinoma. J Am Coll Surg 2004;198:737-41.
40. Itzjowitz S, Kim Y. New carbohydrate tumour markers. Gastroenterology 1986;90:491-4.
41. Jagannath P, Dhir V, Shrikhande S, et al. Effect of preoperative biliary stenting on immediate outcome after pancreaticoduodenectomy. Br J Surg 2005;92:356-61.

he American Journal of Surgery (2009) 197, 466–472

The American Journal of Surgery®

Clinical Surgery-International

Preoperative platelet-lymphocyte ratio is an independent significant prognostic marker in resected pancreatic ductal adenocarcinoma

Richard A. Smith, M.R.C.S.[a,*], Lorraine Bosonnet, P.G.Dip.[a], Michael Raraty, F.R.C.S.[a], Robert Sutton, F.R.C.S.[a], John P. Neoptolemos, F.R.C.S.[a], Fiona Campbell, F.R.C.Path.[b], Paula Ghaneh, F.R.C.S.[a]

[a]Division of Surgery and Oncology, School of Cancer Studies, Royal Liverpool University Hospital, 5th Floor UCD Building, Daulby St., Liverpool, L69 3GA, UK; [b]Department of Pathology, School of Cancer Studies, Royal Liverpool University Hospital, Liverpool, UK

KEYWORDS:
Pancreatic cancer;
Platelets;
Lymphocytes;
Prognostic

Abstract

BACKGROUND: The objective of this study was to investigate whether the preoperative platelet-lymphocyte (P/L) ratio represents a significant prognostic index in resected pancreatic ductal adenocarcinoma.

METHODS: A total of 110 patients undergoing pancreatoduodenectomy for pancreatic ductal adenocarcinoma over a 10-year period were identified from a prospectively maintained database.

RESULTS: The preoperative P/L ratio was found to be a more significant prognostic marker ($P < .001$) than either the lymphocyte count ($P = .007$) or platelet count ($P = .068$) on univariate Cox survival analysis. The median overall survival in patients with a P/L ratio of 150 or less (n = 48) was 19.7 months, 13.7 months in those with a P/L ratio of 151 to 300 (n = 43), and 5.8 months in patients with a value of greater than 300 (n = 19) (log-rank, $P = .006$). The preoperative P/L ratio retained significance on multivariate analysis ($P < .001$), along with tumor size ($P = .010$) and lymph node ratio ($P = .013$).

CONCLUSIONS: The preoperative P/L ratio represents a significant independent prognostic index in patients of resected pancreatic adenocarcinoma.

Of the 10% to 15% of patients who present with operable pancreatic cancer, median survival after resection typically remains in the region of 12 to 18 months and less than 15% of these patients can expect to live beyond 5 years.[1] With adjuvant chemotherapy, 5-year survival rates of more than 20% have been achieved in recent randomized trials.[2,3]

Several histologic parameters of the resected pancreatic cancer specimen have been shown consistently to represent significant prognostic variables.[4–10] These include tumor size, regional lymph node involvement (including lymph node 8a status[7] and lymph node ratio[8–10]), tumor differentiation, and resection margin status. An increasing number of molecular markers also have been identified as having potential prognostic value in resected pancreatic

* Corresponding author. Tel.: +00-44-0151-7063898; fax: +00-44-0151-7065826.
E-mail address: rsmith3@liverpool.ac.uk

Manuscript received October 10, 2007; revised manuscript December 5, 2007

0002-9610/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.amjsurg.2007.12.057

ancer.[11,12] Whether histologic or biological in nature, hese parameters are invariably only amenable to assessment after surgery.

Several studies have investigated potential prognostic ndices that might be amenable to assessment preoperatively. The preoperative neutrophil-lymphocyte ratio has een shown to be a significant prognostic index in resected olorectal cancer.[13] Only 2 previous articles have investigated the potential prognostic value of decreased preoperative lymphocyte counts in resected pancreatic cancer,[14,15] owever, both of these studies were based on limited patient umbers (14 and 23 patients, respectively) and included atients with mixed pancreatic pathology.

An association between the preoperative platelet count nd survival also has been described in resected pancreatic denocarcinoma. Conflicting evidence exists as to whether a reater platelet count contributes to a poorer or more favorble survival outcome,[16–18] however, increased platelet ounts have been shown to be associated with poorer survival in other gastrointestinal malignancies.[19,20]

It was the objective of this study to identify whether the reoperative lymphocyte and platelet counts can provide neaningful prognostic information in resected pancreatic uctal adenocarcinoma by analyzing each parameter as a ontinuous covariate and identifying whether a combination f these 2 parameters, the platelet-lymphocyte (P/L) ratio, ight represent a more useful prognostic index.

Methods

Patients who had a partial pylorus-preserving pancreatoduodenectomy or classic Kausch-Whipple resection rom January 1997 to September 2006 were identified from prospectively maintained database. Only histologically onfirmed patients of pancreatic ductal adenocarcinoma vere included in the analysis. Data collected included patient demographics, surgical details, preoperative full blood ount, details of intervention for preoperative biliary drainge, and standard histologic tumor characteristics. Histoathology reporting was undertaken according to the Royal College of Pathologist's minimum data set for ancreatoduodenectomy reporting.[21] These guidelines tipulate that a positive margin is defined as the presence f at least one cancer cell within 1 mm of any 1 of 7 esection margins on microscopic examination (transection, posterior, medial [superior mesenteric vein], anterior capsule, distal duodenal, proximal duodenal [or gastric], and bile duct). These histopathology reporting tandards were in place at our institution for the entire uration of the study period. Hospital computer records vere used to identify dates of death along with dates of ast clinic attendance for censored patients (ie, patients till alive as of September 2006).

Statistical analysis

Continuous data were analyzed using median, interquartile range, and 95% confidence intervals with 2-tailed Mann-Whitney *U* testing for comparative analysis. The chi-square and the Fisher exact tests were used to analyze categoric data and correlation between 2 continuous data sets was analyzed using the Spearman rank correlation. A preliminary univariate survival analysis was undertaken using Cox proportional hazards regression for each of the hematologic parameters of interest. These were modeled as continuous covariates on both univariate and multivariate analysis to more reliably describe their prognostic value. Kaplan-Meier curves were generated to illustrate survival trends according to P/L ratios. Patients who died within 30 days of surgery (3 of 132 resections) were excluded from survival analyses. Statview version 5 (SAS Institute, Cary, NC) and Microsoft Excel (Microsoft Office 2002; Microsoft Inc., Redmond, WA USA) were used to perform the various statistical functions.

Results

There were 532 patients who had surgical intervention for periampullary tumors during the study period. Of the 297 patients who had partial pancreaticoduodenectomy, 132 patients were identified with histologically confirmed pancreatic ductal adenocarcinoma. Of these, the preoperative full blood count was available in 110 and the results ob-

Table 1 Demographics and preoperative hematology results from resected pancreatic ductal adenocarcinoma patients

No. of patients analyzed	110
Male:female ratio	65:45
Median age, y (interquartile range)	67 (61–73)
Mean interval from FBC to surgery, d (±SEM)	2.4 (.4)
Timing of preoperative FBC	
Number of patients within 24 hours of surgery	75
Within 1–2 days of surgery	17
Within 3–7 days of surgery	12
>7 days of surgery	6
Neutrophilia present (>7.5 × 10^6/mL)	
No	87 (79%)
Yes	23 (21%)
Lymphocytopenia present (<1.0 × 10^6/mL)	
No	102 (93%)
Yes	8 (7%)
Thrombocytosis present (>400 × 10^6/mL)	
No	85 (77%)
Yes	25 (23%)
Intervention for preoperative biliary drainage	
No	18 (16%)
ERCP + stent	85 (77%)
PTC ± stenting	7 (7%)

ERCP = endoscopic retrograde cholangiopancreatography; FBC = full blood count; PTC = percutaneous transhepatic cholangiography; SEM = standard error.

Table 2 Univariate survival analysis of preoperative hematologic parameters as prognostic covariates in resected pancreatic ductal adenocarcinoma (Cox proportional hazards)

	Median value (interquartile range)	Hazard ratio (95% CI)	*P*
Lymphocyte count, $\times 10^6$/mL	1.9 (1.3–2.4)	.677 (.511–.897)	.007
Neutrophil count, $\times 10^6$/mL	5.5 (4.0–7.1)	1.038 (.956–1.127)	.373
Platelet count, $\times 10^6$/mL	303 (258–375)	1.002 (1.000–1.004)	.068
N/L ratio	2.9 (1.9–4.8)	1.047 (.985–1.113)	.140
P/L ratio	159 (116–230)	1.004 (1.002–1.006)	.0001

Hazard ratios for continuous data reflect an increase in the relative risk of death with each incremental increase in a covariate value of 1 unit.
N/L ratio = neutrophil-lymphocyte ratio.

ained nearest to the date of surgery were used for analysis. Demographic and hematologic data are shown in Table 1.

There was a significant inverse relationship between the preoperative neutrophil and lymphocyte counts (rho, −.191; 95% confidence interval [CI], −.345 to .020; P = .040). A significant association between platelets and neutrophils also was observed (rho, .287; 95% CI, .105–.450; P = .003).

The median overall survival recorded in the group of 110 patients analyzed was 13.9 months (95% CI, 12.3–17.0 mo). There were 31 censored patients with a median follow-up time of 12.0 months (interquartile range, 7.8–25.5 mo). The results of univariate survival for each of the hematologic parameters are shown in Table 2. Kaplan-Meier cumulative survival curves for patients stratified into 3 groups according to the preoperative P/L ratio are shown in Fig. 1. Patients with a P/L ratio of greater than 300 had a significantly poorer median survival (5.8 mo; 95% CI, 5.2–10.0) when compared with patients with a P/L ratio of 151 to 300 (13.7 mo; 95% CI, 10.4–16.7) or 150 or less (19.7 mo; 95% CI, 13.3–26.5) (P = .006). Median survival times associated with histologic subgroups are shown in Table 3.

Patients with greater P/L ratios showed an increased likelihood of poorly differentiated tumors (P = .016), however, there was no significant association with any other histologic tumor characteristics (Table 4). The patient group with a P/L ratio greater than 300 had a significantly lower proportion of patients that underwent intervention for preoperative biliary drainage than the other 2 patient groups. A separate survival analysis was undertaken to identify whether preoperative intervention for biliary drainage conferred any survival advantage or disadvantage in the overall patient group. The median survival of 92 patients who underwent biliary drainage in the preoperative period was 13.9 months (95% CI, 12.3–19.7 mo) whereas the median survival of 18 patients who did not undergo biliary drainage was 13.7 months (95% CI, 5.7–24.1 mo) (log rank, P = .873).

The results of univariate and multivariate survival analyses modeling the preoperative P/L ratio as a continuous covariate alongside established histologic prognostic parameters are shown in Table 5. The results showed that the

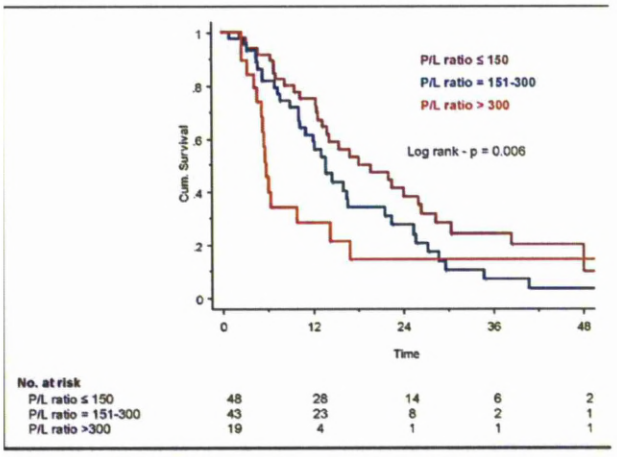


Figure 1 Kaplan-Meier cumulative survival curves for pancreatic ductal adenocarcinoma patients according to the preoperative P/L ratio. Purple line, P/L ratio ≤150; blue line, P/L ratio = 151–300; red line, P/L ratio >300. Log-rank P = .006.

Table 3 Median survival times according to P/L ratio and histologic subgroups

Variable	Median overall survival, mo (95% CI)	*P* (log-rank)
P/L ratio		
≤150 (n = 48)	19.7 (13.3–26.5)	.006
151–300 (n = 43)	13.7 (10.4–16.7)	
>300 (n = 19)	5.8 (5.2–10.0)	
Size*		
≤20 mm (n = 27)	22.5 (12.1–34.9)	.041
>20 mm (n = 81)	13.3 (10.4–16.6)	
Nodal involvement		
Negative (n = 21)	16.7 (13.2–NR)	.042
Positive (n = 89)	13.7 (10.4–17.0)	
Resection margin status		
Negative (n = 32)	22.5 (10.8–34.2)	.059
Positive (n = 77)	13.3 (11.4–15.2)	
Tumor differentiation*		
Well (n = 16)	22.6 (12.5–NR)	.129
Moderate (n = 56)	14.3 (12.3–19.7)	
Poor (n = 37)	12.6 (5.7–16.6)	

NR = not reached.
*Histologic data were incomplete for one patient. Because of the small number of well-differentiated tumors, well and moderately differentiated tumors were grouped together for analysis.

Table 4 Distribution of histologic tumor characteristics stratified by the preoperative P/L ratio

	Number of patients stratified by P/L ratio				Median P/L ratio (interquartile range)
	≤150	151–300	>300	*P* value	
Tumor size					
≤20 mm	13	11	3	.614	151 (110–189)
>20 mm	34	31	16		162 (116–252)
Nodal status					
Negative	7	11	3	.401	179 (140–275)
Positive	41	32	16		155 (114–229)
Resection margin					
Negative	16	14	2	.143	150 (110–193)
Positive	31	29	17		173 (124–291)
Differentiation*					
Well	10	6	0	.016	137 (109–173)
Moderate	24	21	11		156 (116–235)
Poor	13	16	18		187 (139–295)
Adjuvant therapy					
No	34	32	1	.081	177 (116–259)
Yes	14	11	18		149 (107–203)
Preoperative biliary drainage					
No	7	4	7	.035	159 (113–361)
Yes	41	39	12		159 (116–220)

P values for the Fisher exact test calculated were for 3 × 2 contingency tables (ie, Freeman-Halton test).
*χ^2 for 3 × 3 table with Yates correction.

P/L ratio remains a significant independent prognostic marker along with the tumor size and lymph node ratio. Hazard ratios for continuous variables included in a Cox model reflect the proportional increase in relative risk of death with each incremental increase in the continuous prognostic variable of 1 unit. The hazard ratio of 1.004 quoted for the preoperative P/L ratio reflects a regression coefficient of .004 (ie, $e^{.004} = 1.004$). **Therefore, the relative hazard associated with an increase in the P/L ratio by 200 units would be $e^{(200 \times .004)} = 2.226$. The chi-square statistic gives a further indication of the strength of the relationship between the prognostic variable and survival.**

Comments

Systemic inflammation is associated with the release of a number of inhibitory immunologic mediators, most notably interleukin-10 (IL-10) and transforming growth factor-β, which can result in a significant immunosuppressive effect with consequent impaired lymphocyte function.[22] Pancreatic cancer cells directly secrete these 2 inhibitory cytokines[23] and decreased serum levels of transforming growth factor-β2 have been shown to be associated with a more favorable survival outcome in pancreatic ductal adenocarcinoma.[24] Lymphocytopenia has been shown previously to

Table 5 Univariate and multivariate (Cox proportional hazards) survival analysis for prognostic factors in pancreatic ductal adenocarcinoma

	Univariate analysis	Multivariate analysis (n = 104)		
Prognostic factors	*P* value	Hazard ratio (95% CI)	Chi-square	*P* value
Continuous covariates				
Platelet-lymphocyte ratio (n = 110)*	.0001	1.004 (1.002–1.006)	14.092	.0003
Tumor size (n = 108)*	.003	1.025 (1.006–1.044)	6.214	.010
Lymph node ratio (n = 107)*	.004	6.109 (1.465–25.478)	6.508	.013
Categoric covariates				
Resection margin status				
Negative (n = 32)	.062	1.158 (.601–2.233)	.071	.661
Positive (n = 77)				
Tumor differentiation				
Well/moderate (n = 72)	.141	1.186 (.706–1.990)	1.209	.520
Poor (n = 37)				

Histologic data were incomplete for some patients, hence the overall number of patients included in the final Cox model was 104.
*Modeled as continuous covariates on both univariate and multivariate analyses— hazard ratios for continuous data reflect an increase in the relative risk of death with each incremental increase in a covariate value of 1 unit.

e associated more strongly with pancreatic adenocarcinoma when compared with gastric and colorectal cancer,[25] ıggesting that pancreatic malignancy is associated with a ıore marked host inflammatory response than other gastroıtestinal cancers. In addition to pancreatic cancer comıonly showing reduced circulating lymphocyte populaons, a reduced number of tumor-infiltrating lymphocytes ı resected pancreatic adenocarcinoma specimens also have een found to be associated with poorer survival rates after ırgery.[26] Lymphocyte trapping within peritumoral fibrous ssue is believed to be an additional factor by which panreatic cancer cells evade immune surveillance.[27]

Pancreatic cancer commonly causes a hypercoagulable :ate resulting in a predisposition to thromboembolic vents.[28] This is largely attributable to tumor expression of ssue factor that binds to factor VIIa, thereby activating the lotting cascade and promoting thrombin production.[29] Panreatic cancer shows significant overexpression of tissue ıctor when compared with normal pancreatic tissue along /ith up-regulation of vascular endothelial growth factor xpression, thereby potentiating tumor angiogenesis.[30] Tisue factor expression also has been linked with an adverse rognosis in pancreatic ductal adenocarcinoma.[31]

The significance of tumor-platelet interactions within ıis context is incompletely understood. A number of proinammatory mediators (notably IL-1, IL-3, and IL-6) are nown to stimulate megakaryocyte proliferation,[32,33] therebre, the association between a relative thrombocytosis and dverse overall survival in pancreatic cancer might be exlained on the basis that the platelet count reflects an addiional index of systemic inflammation elicited by the tumor. 'latelet aggregation and degranulation along with the conequent release of platelet-derived proangiogenic mediators /ithin the microvasculature of the tumor also could be an nportant determinant of tumor growth.[34] It has been sugested previously that antiplatelet agents might have an ıhibitory effect on the invasive potential of pancreatic ancer cells in vitro by down-regulating tumor secretion of ıatrix metalloproteinase-9.[35]

The preoperative systemic host immune response as a ›rognostic factor in resected pancreatic cancer previously ıas not been evaluated extensively. It has been reported that . more marked preoperative and postoperative systemic nflammatory response (as evidenced by an increased serum -reactive protein [CRP] level >10 mg/L) is associated vith a poorer survival after resection for pancreatic ductal denocarcinoma.[36] Increased preoperative CRP levels also ıave been shown to be associated with poorer survival after urgery in other gastrointestinal malignancies.[37,38] In addiion to an increased CRP level, the presence of a neutro›hilia and relative lymphocytopenia are recognized features ›f the systemic inflammatory response. Only 2 small studies o date have investigated the potential prognostic role of ›reoperative lymphocytopenia in resected pancreatic can:er.[14,15] A similarly small number of studies have investi;ated the potential utility of the preoperative platelet count as a prognostic marker in resected pancreatic cancer and the results from these studies have been conflicting.[16–18]

The present study provides further evidence to support the assertion that the preoperative lymphocyte and platelet counts appear to confer significant prognostic information in resected pancreatic ductal adenocarcinoma. The expected inverse correlation between neutrophil and lymphocyte counts was observed, suggesting that a significant proportion of patients with pancreatic ductal adenocarcinoma show some degree of systemic inflammation before surgery.[36] Furthermore, the positive correlation between neutrophil and platelet count suggests that the preoperative platelet count does reflect an additional index of systemic inflammation. This is perhaps also evidenced by the fact that a preoperative neutrophilia and thrombocytosis were recorded in a similar proportion of the overall patient group.

The results of the preliminary univariate survival analysis indicated that the preoperative lymphocyte count carried the most significant prognostic information of the 3 recorded hematologic parameters when modeled as a continuous covariate, with the platelet count displaying only borderline significance. The P/L ratio was a superior prognostic marker when compared with either individual parameter or the neutrophil-lymphocyte ratio. When categorizing the overall number of patients into 3 groups according to the preoperative P/L ratio, Kaplan-Meier analysis also showed a consistent pattern of progressively poorer survival associated with larger P/L ratios. The median survival associated with a value of greater than 300 appeared to be comparable with what would be expected for locally advanced disease.[39]

Eighty-four percent of patients underwent biliary drainage procedures during the preoperative period, which may represent a potential confounding factor in interpreting the prognostic value of preoperative inflammatory markers. However, a previous meta-analysis has shown that although preoperative biliary stenting increases the risk of early postoperative morbidity such as wound infection,[40] biliary drainage before surgery was shown to have no influence on surgical mortality rates and no literature exists to suggest that stenting has any impact on intermediate or late survival in resected pancreatic cancer. The group with a P/L ratio greater than 300 actually had a significantly smaller proportion of patients undergoing biliary drainage in the preoperative period, with no difference in survival between patients who did or did not require biliary drainage in the overall group. Given these findings (along with the existing literature), it is unlikely that the issue of preoperative biliary drainage is a significant factor in explaining the strong association between the preoperative host inflammatory response (as measured by the P/L ratio) and overall postoperative survival shown in the present article.

Similarly, there was no significant difference in the proportion of patients who went on to receive adjuvant therapy in each of the 3 groups stratified by the P/L ratio. The results are consistent with the hypothesis that greater preoperative P/L ratios reflect an enhanced host inflammatory response to

ıore aggressive tumor biology. When stratifying patients ccording to histologic characteristics (Table 3), a clear end towards poorly differentiated tumors showing greater /L ratios was observed.

Rather than analyze the P/L ratio as a categorized covariate, the multivariate analysis was conducted using connuous covariates where possible to maximize the statistical alidity of the analysis. This approach avoids the potential ignificant bias associated with categorizing continuous data or prognostic purposes.[41] The P/L ratio emerged as the ıost significant determinant of survival whereas tumor size nd the lymph node ratio also retained significance. Conucting the multivariate analysis in this way also allows for more meaningful comparison of individual prognostic ıdices to be made when comparing results between differnt studies.

The powerful preoperative prediction of survival by the P/L ıtio merits validation in a larger patient cohort and requires omparison with other potential prognostic markers such as a arbohydrate antigen (CA19-9)[42] and CRP.[36] This may have articular implications for developing criteria for more selecve use of laparoscopic staging along with stratification of atients in future trials for neoadjuvant and adjuvant therapy.

ıcknowledgments

The authors are grateful to Dr. Catrin Tudur-Smith, PhD, enior Lecturer in Medical Statistics, School of Cancer tudies, University of Liverpool, for statistical advice.

Supported by Cancer Research UK (registered charity o. 1089464).

leferences

1. Alexakis N, Halloran C, Raraty M, et al. Current standards of surgery for pancreatic cancer. Br J Surg 2004;91:1410–27.
2. Neoptolemos JP, Stocken DD, Friess H, et al. A randomized trial of chemotherapy after resection of pancreatic cancer. N Engl J Med 2004;350:1200–10.
3. Oettle H, Post S, Neuhaus P, et al. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 2007;297:267–77.
4. Neoptolemos JP, Stocken DD, Dunn JA, et al. Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. Ann Surg 2001;234:758–68.
5. Bassi C, Stocken DD, Olah A, et al. Influence of surgical resection and post-operative complications on survival following adjuvant treatment for pancreatic cancer in the ESPAC-1 randomized controlled trial. Dig Surg 2005;22:353–63.
6. Winter JM, Cameron JL, Campbell KA, et al. 1423 pancreaticoduodenectomies for pancreatic cancer: a single-institution experience. J Gastrointest Surg 2006;10:1199–210.
7. Connor S, Bosonnet L, Ghaneh P, et al. Survival of patients with periampullary carcinoma is predicted by lymph node 8a but not by lymph node 16b1 status. Br J Surg 2004;91:1592–9.
8. Pawlik TM, Gleisner AL, Cameron JL, et al. Prognostic relevance of lymph node ratio following pancreaticoduodenectomy for pancreatic cancer. Surgery 2007;141:610–8.
9. Sierzega M, Popiela T, Kulig J, et al. The ratio of metastatic/resected lymph nodes is an independent prognostic factor in patients with node-positive pancreatic head cancer. Pancreas 2006;33:240–5.
10. Berger AC, Watson JC, Ross EA, et al. The metastatic/examined lymph node ratio is an important prognostic factor after pancreaticoduodenectomy for pancreatic adenocarcinoma. Am Surg 2004;70:235–40.
11. Kim J, Reber HA, Dry SM, et al. Unfavourable prognosis associated with K-ras gene mutation in pancreatic cancer surgical margins. Gut 2006;55:1598–605.
12. Kawesha A, Ghaneh P, Andren-Sandberg A, et al. K-ras oncogene subtype mutations are associated with survival but not expression of p53, p16(INK4A), p21(WAF-1), cyclin D1, erbB-2 and erb-B3 in resected pancreatic ductal adenocarcinoma. Int J Cancer 2000;89:469–74.
13. Walsh SR, Cook EJ, Goulder F, et al. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. J Surg Oncol 2005;91:181–4.
14. Yamaguchi K, Noshiro H, Shimizu S, et al. Long-term and short-term survivors after pancreatectomy for pancreatic cancer. Int Surg 2000;85:71–6.
15. Fogar P, Sperti C, Basso D, et al. Decreased total lymphocyte counts in pancreatic cancer: an index of adverse outcome. Pancreas 2006;32:22–8.
16. Schwarz RE, Keny H. Preoperative platelet count predicts survival after resection of periampullary adenocarcinoma. Hepatogastroenterology 2001;48:1493–8.
17. Suzuki K, Aiura K, Kitagou M, et al. Platelet counts closely correlate with the disease-free survival interval of pancreatic cancer patients. Hepatogastroenterology 2004;51:847–53.
18. Brown KM, Domin C, Aranha GV, et al. Increased preoperative platelet count is associated with decreased survival after resection for adenocarcinoma of the pancreas. Am J Surg 2005;189:278–82.
19. Shimada H, Oohira G, Okazumi S, et al. Thrombocytosis associated with poor prognosis in patients with oesophageal carcinoma. J Am Coll Surg 2004;198:737–41.
20. Monreal M, Fernandez-Llamazares J, Pinol M, et al. Platelet count and survival in colorectal cancer—a preliminary study. Thromb Haemost 1998;79:916–8.
21. Campbell F, Bennett M, Foulis AJ. Minimum dataset for histopathological reporting of pancreatic, ampulla of vater and bile duct carcinoma. Royal Coll Pathol 2002. Available: http://www.rcpath.org. Accessed October 9, 2007.
22. Salazar-Onfray F, Lopez MN, Mendoza-Naranjo A. Paradoxical effects of cytokines in tumor immune surveillance and tumor escape. Cytokine Growth Factor Rev 2007;18:171–82.
23. Bellone G, Turletti A, Artusio E, et al. Tumor-associated transforming growth factor-beta and interleukin-10 contribute to a systemic Th2 immune phenotype in pancreatic carcinoma patients. Am J Pathol 1999;155:537–47.
24. Bellone G, Smirne C, Angelo F, et al. Cytokine expression in human pancreatic carcinoma cells in surgical specimens: implications for survival. Cancer Immunol Immunother 2006;55:684–98.
25. Romano F, Uggeri F, Crippa S, et al. Immunodeficiency in different histotypes of radically operable gastrointestinal cancers. J Exp Clin Cancer Res 2004;23:195–200.
26. Fukunaga A, Miyamoto M, Yasushi C, et al. CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. Pancreas 2004;28:26–31.
27. von Bernstorff W, Voss M, Freichel S, et al. Systemic and local immunosuppression in pancreatic cancer patients. Clin Cancer Res 2001;7(Suppl):925s–32s.
28. Khorana AA, Fine RL. Pancreatic cancer and thromboembolic disease. Lancet Oncol 2004;5:655–63.

9. Haas SL, Jesnowski R, Steiner M, et al. Expression of tissue factor in pancreatic adenocarcinoma is associated with activation of coagulation. World J Gastroenterol 2006;12:4843–9.
0. Khorana AA, Ahrendt SA, Ryan CK, et al. Tissue Factor expression, angiogenesis and thrombosis in pancreatic cancer. Clin Cancer Res 2007;13:2870–5.
1. Nitori N, Ino Y, Nakanishi T, et al. Prognostic significance of tissue factor in pancreatic ductal adenocarcinoma. Clin Cancer Res 2005;11: 2531–9.
2. Klinger MH, Jelkmann W. Role of blood platelets in infection and inflammation. J Interferon Cytokine Res 2002;22:913–22.
3. Alexandrakis MG, Passam FH, Moschandrea IA, et al. Levels of serum cytokines and acute phase proteins in patients with essential and cancer-related thrombocytosis. Am J Clin Oncol 2003;26:135–40.
4. Sierko E, Wojtukiewicz MZ. Platelets and angiogenesis in malignancy. Semin Thromb Hemost 2004;30:95–108.
5. Suzuki K, Aiura K, Ueda M, et al. The influence of platelets on the promotion of invasion by tumor cells and inhibition by antiplatelet agents. Pancreas 2004;29:132–40.
6. Jamieson NB, Glen P, McMillan DC, et al. Systemic inflammatory response predicts outcome in patients undergoing resection for ductal adenocarcinoma head of pancreas. Br J Cancer 2005;92:21–3.
37. Canna K, McArdle PA, McMillan DC, et al. The relationship between tumour T-lymphocyte infiltration, the systemic inflammatory response and survival in patients undergoing curative resection for colorectal cancer. Br J Cancer 2005;92:651–4.
38. Crumley ABC, McMillan DC, McKernan M, et al. An elevated C-reactive protein concentration, prior to surgery, predicts poor cancer-specific survival in patients undergoing resection for gastro-oesophageal cancer. Br J Cancer 2006;94:1568–71.
39. Sultana A, Tudor-Smith C, Cunningham D, et al. Systematic review, including meta-analysis, on the management of locally advanced pancreatic cancer using radiation/combined modality therapy. Br J Cancer 2007;96:1183–90.
40. Sewnath ME, Karsten TM, Prins MH, et al. A meta-analysis on the efficacy of preoperative biliary drainage for tumors causing obstructive jaundice. Ann Surg 2002;236:17–27.
41. Altman DG, Lausen B, Sauerbrei W, et al. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. J Natl Cancer Inst 1994;86:829–35.
42. Kau SY, Shyr YM, Su CH, et al. Diagnostic and prognostic values of CA19-9 and CEA in periampullary cancers. J Am Coll Surg 1999; 188:415–20.

Histopathology 2009, 55, 277–283. DOI: 10.1111/j.1365-2559.2009.03376.x

Classification of R1 resections for pancreatic cancer: the prognostic relevance of tumour involvement within 1 mm of a resection margin

Fiona Campbell, Richard A Smith,[1] Philip Whelan,[1] Robert Sutton,[1] Michael Raraty,[1] John P Neoptolemos[1] & Paula Ghaneh[1]

Department of Pathology and [1]Division of Surgery and Oncology, School of Cancer Studies, University of Liverpool, Royal Liverpool University Hospital, Liverpool, UK

Date of submission 4 November 2008
Accepted for publication 18 February 2009

Campbell F, Smith R A, Whelan P, Sutton R, Raraty M, Neoptolemos J P & Ghaneh P (2009) *Histopathology* **55**, 277–283

Classification of R1 resections for pancreatic cancer: the prognostic relevance of tumour involvement within 1 mm of a resection margin

Aims: The current Royal College of Pathologists guidelines for pancreatoduodenectomy specimen reporting recommend that microscopic evidence of tumour within 1 mm of a resection margin (RM) should be classified as R1. No clinical evidence exists to justify this classification. The aim of this study was to identify the proportion of pancreatoduodenectomy specimens in which 'equivocal' RMs are present (tumour involvement within 1 mm of, but not directly reaching, one or more resection margins) and whether the survival of these patients was similar to that of patients with unequivocal' RM involvement.

Methods and results: Patients with histologically confirmed pancreatic ductal adenocarcinoma undergoing pancreatoduodenectomy between 1997 and 2007 (n = 163) were identified from a prospective database. One hundred and twenty-eight cases (79%) were classified as R1. Of these, 57 (45% of all R1 cases) were based on 'equivocal' margin involvement. There was no significant difference in overall survival between equivocal and unequivocal R1 resections (log rank, P = 0.102). All R1 resections had a poorer survival on univariate (log rank, P = 0.013), but not multivariate, analysis (Cox, P = 0.132).

Conclusions: Our results indicate that cases with microscopic tumour involvement within 1 mm of a resection margin should be considered synonymous with incomplete excision for resected pancreatic cancer.

Keywords: pancreatic cancer, prognosis, resection margin

Abbreviation: CI, confidence interval

Introduction

Pancreatic ductal adenocarcinoma has a poor prognosis and surgical resection remains the only potentially curative intervention. Due to its late presentation and aggressive tumour biology, only 10–15% of cases are resectable.[1] Patients who undergo resection for pancreatic cancer typically have 5-year survival rates of <10%. However, adjuvant chemotherapy has been demonstrated to improve long-term survival outcomes significantly in recent randomized trials.[2,3] Microscopic tumour involvement of the surgical resection margin (R1) represents one of a number of histological features of the resected pancreatic specimen (including tumour size, differentiation and nodal involvement) that have been reported to confer significant prognostic information.[4–9] Quoted R1 resection rates can vary significantly between individual specialist centres (14–85%),[7–10] and it is not known to what extent these differences reflect different pathological practices.

Address for correspondence: Dr F Campbell, Consultant Gastrointestinal Pathologist, Department of Pathology, Royal Liverpool University Hospital, 5th Floor Duncan Building, Daulby Street, Liverpool L69 3GA, UK. e-mail: f.campbell@liv.ac.uk

The Royal College of Pathologists minimum dataset for histological reporting of pancreatoduodenectomy specimens[11] recommends that cases with microscopic evidence of tumour extension to within 1 mm of one or more resection margins should be classified as R1. However, this changes the current definition of R1, which is defined by the International Union Against Cancer as tumour at the resection margin(s). No previous clinical studies have been conducted to demonstrate whether cases with tumour within 1 mm of, but not directly reaching, one or more resection margins (i.e. an 'equivocal' R1 status), have comparable survival outcomes when compared with cases with 'unequivocal' margin involvement (i.e. cancer reaching the resection margin on microscopy). The principal objectives of this study were to identify the proportion of patients undergoing pancreatoduodenectomy for pancreatic ductal adenocarcinoma in whom an R1 classification was based on one or more 'equivocal' resection margins, and to establish whether this distinction conferred any prognostic relevance.

Materials and methods

Consecutive patients with histologically confirmed pancreatic ductal adenocarcinoma undergoing pancreatoduodenectomy at the Royal Liverpool University Hospital between January 1997 and December 2007 were identified from a prospective database. All the histology reports were read to identify cases where margin involvement was documented by the reporting pathologist. In all cases reported as R0, the histopathology slides were retrieved and reviewed by a single consultant pathologist (F.C.) in order to confirm an R0 classification. Figure 1B illustrates an example of an 'equivocal' resection margin where tumour can be seen to extend to within 1 mm, without directly reaching the margin itself. Figure 1C shows an example of direct tumour involvement at a painted resection margin.

Specimens were serially sliced axially[9,10] and histopathology reporting was conducted according to the Royal College of Pathologists minimum dataset for pancreatic and periampullary adenocarcinoma.[11] The reporting criteria in the dataset were routinely used at our institution both before and after their publication in 2002. These guidelines recommend that the status of six discrete resection margins be documented by the reporting pathologist: the pancreatic transection margin, the medial (or superior mesenteric vessel) margin, the posterior margin, the proximal duodenal (or gastric) margin, the distal duodenal margin and the common bile duct margin. Microscopic evidence of tumour involvement at any one of these six margins results in an R1 classification. Isolated tumour involvement of the anterior surface of the pancreatic specimen

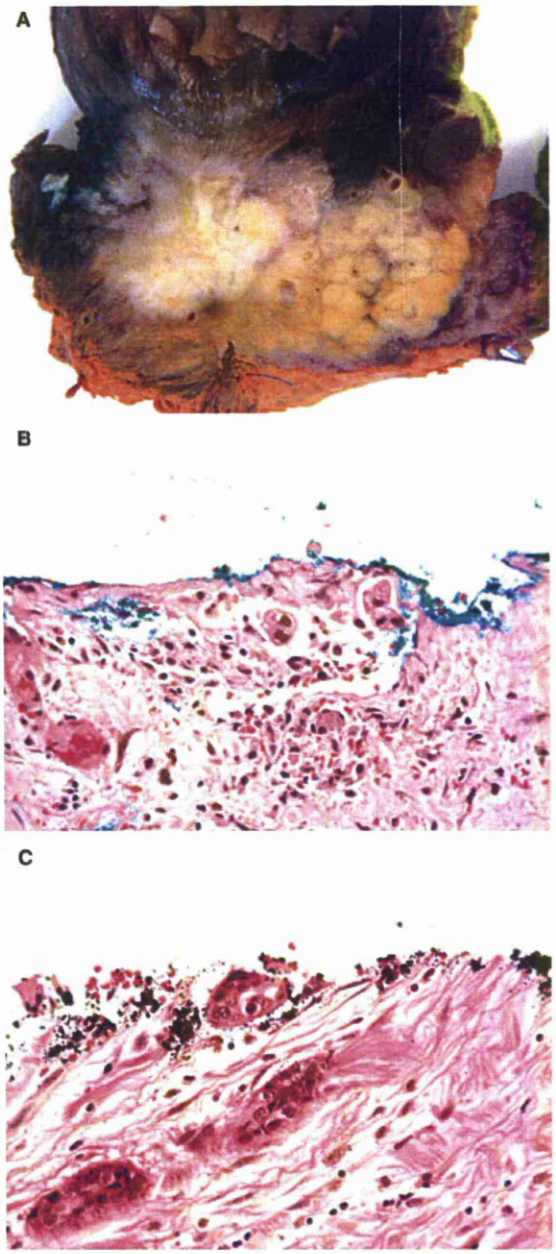


Figure 1. A, Macroscopic photograph shows an axial slice through the head of the pancreas with adenocarcinoma close to the posterior (green) resection margin and clear of the medial (orange) resection margin. A lymph node is seen within the anterior pancreatoduodenal groove (yellow paint). Light micrographs showing an 'equivocal' resection margin (B) with adenocarcinoma extending to within <1 mm of a painted resection margin, and 'unequivocal' resection margin involvement (C).

vas not considered as an R1 resection in our patient ohort. Similarly, the presence of pancreatic intraepihelial neoplasia-3 at an otherwise negative transection nargin was not considered an R1 resection. No cases vere classified as R1 exclusively on the basis of erineural invasion at a resection margin. Similarly, iodal involvement at a resection margin did not onstitute an R1 classification in the absence of direct umour involvement. No R2 resections were included n this series of patients. Survival data were obtained rom hospital computer records. Data regarding adjuvant therapy were also collected. Patient selection for idjuvant therapy was not based on resection margin tatus.

TATISTICS

Continuous data were described using median, interquartile range and 95% confidence intervals (CI). Overall survival was calculated from the date of esection to the date of death. Univariate survival for ategorical data was investigated using Kaplan–Meier nalysis with significance assessed by log rank (Mantel–Cox) testing. Univariate survival for continuous lata was assessed using Cox proportional hazards egression. Multivariate survival analysis was conducted using Cox regression with inclusion of clinicopathological factors of univariate significance. Resection margin status was analysed alongside umour size, lymph node ratio, differentiation and idjuvant chemotherapy. The relationship between istological factors and likelihood of resection margin nvolvement was assessed using logistic regression. Significance was set at $P < 0.05$. Statistical analyses were conducted using STATVIEW version 5 (SAS Institute, Cary, NC, USA).

Results

One hundred and sixty-three patients were identified for whom a pancreatoduodenectomy was performed for pancreatic ductal adenocarcinoma during the study period. Table 1 summarizes the demographic and clinicopathological data for this patient group. Of the 128 cases (79%) classified as R1, 57 (45%) were based on tumour involvement within 1 mm of one or more margins, without direct involvement of the margin itself (i.e. an 'equivocal' margin). Table 2 demonstrates a breakdown of resection margin involvement according to the number of involved margins per specimen and to the distribution of margin involvement. These results indicate that 35% of R1 resections exhibited multifocal margin involvement (i.e. more than one margin involved in a single specimen), while the posterior and medial margins were the most commonly involved margin locations.

Table 1. Demographic and clinicopathological data

Total number of patients	163
Male:female	91:72
Median age (IQR)	66.4 (60.8–73.0) years
30-day mortalities	4 (2%)
Median overall survival (95% CI)	13.9 months (12.4, 16.1)
Adjuvant chemotherapy given	44 (27%)
Type of pancreatoduodenectomy	
Pylorus-preserving	147 (90%)
Classical	16 (10%)
Median tumour size (IQR)	30 (23–38) mm
Tumour differentiation	
Well	25 (15%)
Moderate	84 (52%)
Poor	53 (33%)
Lymph node status	
Positive	25 (15%)
Negative	138 (85%)
Median lymph node yield (IQR)	18 (12–25)
Median lymph node ratio for node-positive cases (IQR)	0.23 (0.14–0.37)
T stage	
T1	7 (4%)
T2	17 (10%)
T3	135 (83%)
T4	4 (3%)
Resection margin status	
Negative	35 (21%)
Positive	128 (79%)
Direct involvement	71
<1 mm	57

IQR, interquartile range.

Table 2. Distribution of resection margin involvement

All R1 resections	128 (79%)
Number of involved resection margins per specimen	
1	83 (65%)
2	37 (29%)
3	7 (5%)
4	1 (1%)
Distribution of resection margin involvement	
Posterior	69 (54%)
Medial	64 (50%)
Transection	38 (30%)
Proximal duodenal/gastric	6 (5%)
Common bile duct	4 (3%)
Distal duodenal	–

THE PROGNOSTIC RELEVANCE OF 'EQUIVOCAL' RESECTION MARGINS

Figure 2A illustrates the Kaplan–Meier survival curves according to the three margin classifications—equivocal R1 (<1 mm), unequivocal R1 (direct) and R0. Equivocal R1 cases had a median survival of 15.4 months (95% CI 11.3, 18.2) compared with 12.6 months (95% CI 9.2, 14.3) for unequivocal R1 cases and 25.4 months (95% CI 10.5, 40.8) for R0 cases. When comparing the overall R1 group with R0 cases (Figure 2B), a significant difference in survival was recorded (log rank, $P = 0.013$). When comparing equivocal with unequivocal R1 cases (Figure 2C), no significant difference in survival was recorded (log rank, $P = 0.102$). This was similarly true when comparing the equivocal R1 group with the R0 group (log rank, $P = 0.114$). Table 3 demonstrates the results of both univariate and multivariate survival analysis using Cox proportional hazards regression. These results indicate that resection margin status failed to maintain significance when analysed alongside the other important histopathological factors (lymph node ratio, tumour size, differentiation) and adjuvant chemotherapy.

PROGNOSTIC RELEVANCE OF MARGIN LOCATION

When analysing only R1 resections, involvement of the transection margin was found to exhibit a marginal trend towards poorer survival. However, this failed to reach significance (log rank, $P = 0.085$). Neither posterior ($P = 0.217$) nor medial margin involvement ($P = 0.257$) was associated with poorer outcomes within the R1 group of patients. When analysing the

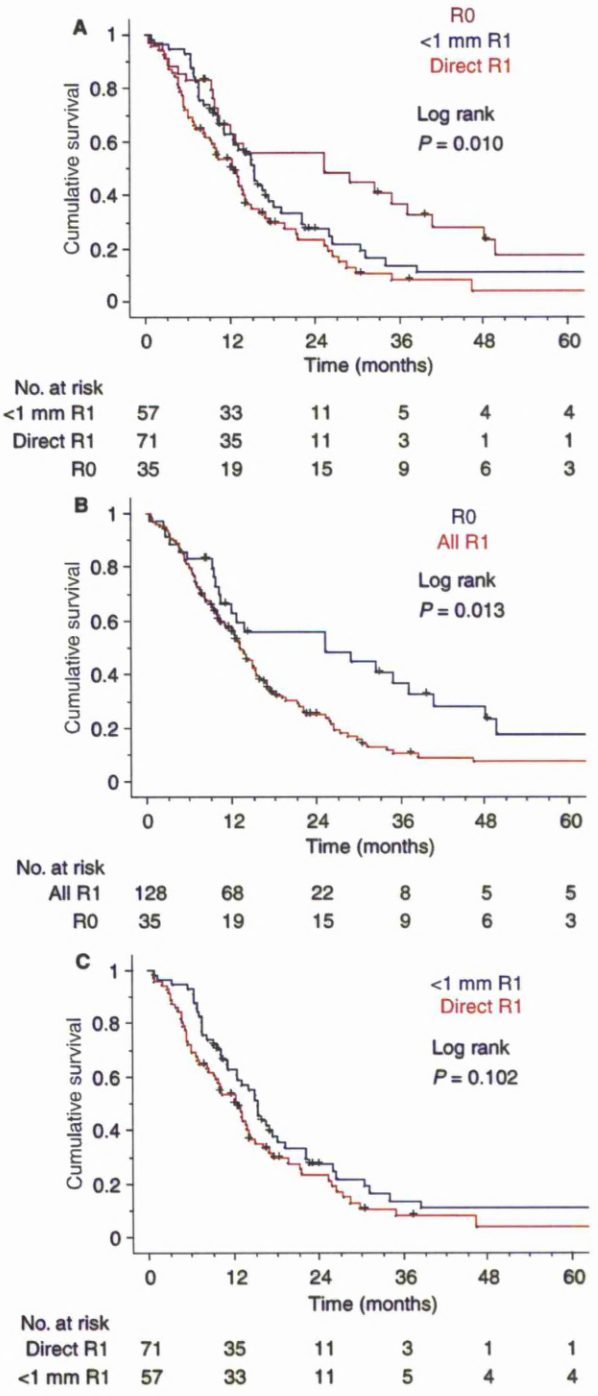


Figure 2. Kaplan–Meier cumulative survival curves according to resection margin status. Crosses on survival curves indicate censored cases. Numbers at risk recorded beneath each *x*-axis.

Table 3. Univariate and multivariate survival analysis for histopathological prognostic variables

	Univariate analysis			Multivariate analysis (n = 155)		
	Hazard ratio (95% CI)	χ^2	P	Hazard ratio (95% CI)	χ^2	P
Tumour size†	1.020 (1.005, 1.036)	6.629	**0.010**	1.016 (1.000, 1.033)	3.887	**0.049**
Poor tumour differentiation*	1.660 (1.152, 2.392)	7.387	**0.007**	1.428 (0.965, 2.112)	1.783	0.075
Lymph node ratio†	4.679 (2.003, 10.930)	12.713	**<0.001**	3.116 (1.304, 7.444)	6.544	**0.011**
Resection margin-positive	1.747 (1.117, 2.734)	5.978	**0.015**	1.443 (0.896, 2.324)	2.272	0.132
Adjuvant chemotherapy	0.583 (0.384, 0.887)	6.363	**0.012**	0.605 (0.394, 0.930)	5.242	**0.022**

*Poor tumour differentiation analysed against well/moderately differentiated tumours. Resection margin status (R1 versus R0) and adjuvant chemotherapy (yes versus no) were also analysed as categorical covariates.

†Tumour size and lymph node ratio were included as continuous covariates in the Cox model (NB. hazard ratios for continuous prognostic data reflect increase in relative hazard with each unit increase in covariate value). Histological data were incomplete for a small number of cases. Hence, the final multivariate model included 155 cases.

$P < 0.05$ highlighted in bold.

group of 35 R0 resections, 12 cases exhibited isolated tumour involvement (either direct or <1 mm) of the anterior pancreatic surface. There was no significant difference in survival between this group of patients and the remaining 23 R0 resections (log rank, $P = 0.220$).

Relationship between R1 status and other histological tumour characteristics

A logistic regression analysis was conducted to identify whether any of the other histological tumour characteristics were associated with an increased likelihood of microscopic margin involvement. R1 likelihood was included as the dependent variable in this analysis. Increasing tumour size (recorded in mm) was associated with a significantly increased likelihood of an R1 resection when included as a continuous independent variable (odds ratio 1.049; 95% CI 1.010, 1.088; $P = 0.013$). Neither poor tumour differentiation ($P = 0.095$) nor nodal status ($P = 0.738$) exhibited a significant relationship with R1 likelihood in this patient cohort.

Discussion

The relative prognostic significance of resection margin status is variably reported for pancreatic cancer. Although several studies have suggested that resection margin involvement has significant prognostic value on multivariate analysis alongside other histological tumour characteristics,[4–6] studies including larger patient series typically demonstrate that R1 status either fails to maintain significance on multivariate analysis[7,12] or that R1 status fails to emerge as a significant univariate predictor of survival.[13,14] These results have also been mirrored in a recent meta-analysis of four adjuvant therapy trials, which failed to demonstrate a significant overall survival difference according to resection margin status in a pooled group of 869 pancreatic adenocarcinoma resections.[15]

Highly variable R1 resection rates for pancreatic cancer are commonly quoted in different studies. Sizeable multicentre adjuvant therapy trials[2,3] have previously reported R1 resection rates of 17–19%. However, these studies do not report potential differences in R1 rates between individual surgical centres. Studies reporting results from single-centre cohorts have demonstrated marked variability in R1 rates (17–85%).[7–10] It is unknown to what extent this heterogeneity in quoted R1 rates may be explained by differences in pathological practice rather than operative expertise. Meaningful comparison of R1 rates between individual centres is further complicated by the lack of standardized terminology for margins, and the lack of any internationally recognized protocol for pathological examination and reporting. However, increasing evidence exists to suggest that the standard of histopathological processing and reporting has a significant impact on R1 resection rates.[9,10]

A previous study by Verbeke *et al.*[10] demonstrated that utilization of a standardized protocol for histological processing and examination of pancreatoduodenectomy specimens for pancreatic cancer, based on the Royal College of Pathologists guidelines, was associated with an R1 resection rate of 85%. This study also

demonstrated a significant correlation between an increasing number of tissue blocks taken and an increasing likelihood of an R1 classification. A more recent study by Esposito *et al.*,[9] using a similar standardized histopathology protocol, reported an R1 rate of 76%. These findings are consistent with the hypothesis that a negative resection margin status may be commonly incorrectly assigned to cases with suboptimal pathological processing. The assertion that R1 resections are commonly under-reported is also supported by the observation that 60–80% of cases with resected pancreatic cancer develop local recurrence,[16–18] a finding that seems inconsistent with quoted R1 resection rates of <20%. Differences in histological R1 classification between individual centres may also in part explain the variable reporting of resection margin status as a prognostic index for pancreatic cancer.

The present study represents the first attempt to quantify the impact of the '<1 mm rule' in defining R1 classification for resected pancreatic cancer and provides further evidence to suggest that R1 resections may be commonly under-reported. Our R1 resection rate of 79% is comparable to the rates quoted by Esposito *et al.* (76%) and Verbeke *et al.* (85%) [9,10] using standardized pathology protocols based on the Royal College of Pathologists guidelines. Our results also suggest a similar proportion of multifocal R1 resections (35%) when compared with these two studies (32% and 45%, respectively). There is currently no evidence to indicate that nodal involvement or perineural/intravascular invasion at a resection margin should constitute an R1 classification. These features reflect distinct infiltrative tumour characteristics that are reported separately and represent discrete prognostic factors. Therefore, incorporating these additional features into a system of resection margin classification potentially duplicates prognostic information. The presence of nodal involvement or perineural/intravascular invasion at a resection margin did not constitute an R1 case in this study. It has also previously been demonstrated that this issue is relevant for only a small proportion of cases.[9]

Our findings indicate that the '<1 mm rule' has a significant impact on the quoted R1 resection rate. In total, 45% of all R1 resections in this cohort of patients were based on 'equivocal' margin involvement (i.e. tumour within 1 mm of one or more margins, in the absence of direct involvement). If these cases had been classified as R0, the R1 resection rate would have fallen significantly from 79 to 44%. Analysis of the survival curves supports the recommendation made in the Royal College of Pathologists guidelines that tumour involvement within 1 mm of a resection margin should be considered synonymous with incomplete excision. Although the difference between equivocal R1 cases and R0 cases did not reach significance, the median survival of the equivocal R1 group (15.4 months) was clearly more comparable to the unequivocal R1 group (12.6 months), when compared with the R0 group (25.4 months). Kaplan–Meier analyses also demonstrate that the survival distribution for equivocal R1 cases exhibits much closer overall concordance with the unequivocal R1 group. The overall survival pattern raises the possibility that a larger study may result in the equivocal R1 cases representing an intermediate prognostic group. However, a case for pathological subcategorization of R1 resections could be supported only if a clear distinction between the two R1 groups was evident, which was not demonstrated from the present data. Larger studies are required to address this issue definitively.

When analysing the distribution of margin involvement in R1 resections for pancreatic cancer, the finding that the posterior and medial margins represent the most frequently involved margin locations is also consistent with the existing literature.[9,10,19] The proportion of cases with microscopic tumour involvement of the transection margin in the present study is greater than quoted elsewhere.[9,10] However, the majority of these cases typically exhibited very focal involvement, and only nine cases exhibited isolated transection margin involvement. The anterior surface of the pancreas is not considered to be a surgical margin and was therefore not considered as an R1 resection in this study. However, involvement of this surface could potentially affect survival. When analysing the small subgroup of patients with isolated tumour involvement of the anterior surface (whether direct or <1 mm), there was no significant difference in survival compared with R0 cases.

Previous studies have suggested that the presence of poor tumour differentiation[20] and increasing tumour size[7] may be associated with an increased likelihood of resection margin involvement in pancreatic cancer. The results from the present study are consistent with these findings. However, this observation reached significance only for tumour size. The association between these histological tumour characteristics and R1 likelihood may also, in part, explain why resection margin status commonly fails to emerge as a significant independent prognostic index when analysed in a multivariate context, as in the present study.

In summary, this study has provided the first clinical evidence to support the Royal College of Pathologists guidelines regarding R1 classification in pancreatoduodenectomy specimens for pancreatic cancer. The find-

ngs highlight the importance of standardized histo-
›athological reporting and provide a potential expla-
ıation for the significant heterogeneity in reported R1
·ates quoted by different specialist cancer centres. The
lata also provide further evidence to indicate that
·obust pathological practice is a more important
leterminant of R1 classification in pancreatic cancer
han operative expertise. These findings have consid-
›rable implications for stratification of patients as part
›f future adjuvant therapy trials, including the pending
ESPAC-3 results.[21]

Acknowledgement

This study was supported by Cancer Research UK.

References

1. Alexakis N, Halloran C, Raraty M, Ghaneh P, Sutton R, Neoptolemos JP. Current standards of surgery for pancreatic cancer. *Br. J. Surg.* 2004; **91**; 1410–1427.
2. Neoptolemos JP, Stocken DD, Friess H *et al.* A randomized trial of chemotherapy after resection of pancreatic cancer. *N. Engl. J. Med.* 2004; **350**; 1200–1210.
3. Oettle H, Post S, Neuhaus P *et al.* Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA* 2007; **297**; 267–277.
4. Benassi G, Mastrorilli M, Quarto G *et al.* Factors influencing survival after resection for ductal adenocarcinoma of the head of pancreas. *J. Surg. Oncol.* 2000; **73**; 212–218.
5. Han S, Jang J, Kim S, Kim WH, Lee KU, Park YH. Analysis of long-term survivors after surgical resection for pancreatic cancer. *Pancreas* 2006; **32**; 271–275.
6. Moon HJ, An JY, Heo JS, Choi SH, Joh JW, Kim YI. Predicting survival after surgical resection for pancreatic ductal adenocarcinoma. *Pancreas* 2006; **32**; 37–43.
7. Raut CP, Tseng JF, Sun CC *et al.* Impact of resection status on pattern of failure and survival after pancreaticoduodenectomy for pancreatic adenocarcinoma. *Ann. Surg.* 2007; **246**; 52–60.
8. Winter JM, Cameron JL, Campbell KA *et al.* 1423 pancreaticoduodenectomies for pancreatic cancer: a single-institution experience. *J. Gastrointest. Surg.* 2006; **10**; 1199–1210.
9. Esposito I, Kleeff J, Bergmann F *et al.* Most pancreatic cancer resections are R1 resections. *Ann. Surg. Oncol.* 2008; **15**; 1651–1660.
10. Verbeke CS, Leitch D, Menon KV, McMahon MJ, Guillou PJ, Anthoney A. Redefining the R1 resection in pancreatic cancer. *Br. J. Surg.* 2006; **93**; 1232–1237.
11. Campbell F, Bennett M, Foulis AJ. *Minimum dataset for histopathological reporting of pancreatic, ampulla of Vater and bile duct carcinoma.* London: Royal College of Pathologists, 2002. Available at http://www.rcpath.org. Last accessed on 27 October 2008.
12. Pawlik TM, Gleisner AL, Cameron JL *et al.* Prognostic relevance of lymph node ratio following pancreaticoduodenectomy for pancreatic cancer. *Surgery* 2007; **141**; 610–618.
13. Bassi C, Stocken DD, Olah A *et al.* Influence of surgical resection and post-operative complications on survival following adjuvant treatment for pancreatic cancer in the ESPAC-1 randomized controlled trial. *Dig. Surg.* 2005; **22**; 353–363.
14. Jarufe NP, Coldham C, Mayer AD, Mirza DF, Buckels JAC, Bramhall SR. Favourable prognostic factors in a large UK experience of adenocarcinoma of the head of the pancreas and periampullary region. *Dig. Surg.* 2004; **21**; 202–209.
15. Butturini G, Stocken DD, Wente MN *et al*; Pancreatic Cancer Meta-Analysis Group. Influence of resection margins and treatment on survival in patients with pancreatic cancer: meta-analysis of randomized controlled trials. *Arch. Surg.* 2008; **143**; 75–83.
16. Kayahara M, Nagakawa T, Ueno K, Ohta T, Takeda T, Miyazaki I. An evaluation of radical resection for pancreatic cancer based on the mode of recurrence as determined by autopsy and diagnostic imaging. *Cancer* 1993; **72**; 2118–2223.
17. Westerdahl J, Andren-Sandberg A, Ihse I. Recurrence of exocrine pancreatic cancer: local or hepatic? *Hepatogastroenterology* 1993; **40**; 384–387.
18. Sperti C, Pasquali C, Piccoli A, Pedrazzoli S. Recurrence after resection for ductal adenocarcinoma of the pancreas. *World J. Surg.* 1997; **21**; 195–200.
19. Nagakawa T, Nagamori M, Futakami F *et al.* Results of extensive surgery for pancreatic carcinoma. *Cancer* 1996; **77**; 640–645.
20. Neoptolemos JP, Stocken DD, Dunn JA *et al*; European Study Group for Pancreatic Cancer. Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. *Ann. Surg.* 2001; **234**; 758–768.
21. Neoptolemos JP, Buchler M, Stocken DD *et al.* ESPAC-3(v2): a multicenter, international, open-label, randomized, controlled phase III trial of adjvant 5-fluorouracil/folinic acid (5-FU/FA) versus gemcitabine (GEM) in patients with resected pancreatic ductal adenocarcinoma. *J. Clin. Oncol.* 2009; **27**; 18 suppl (abstr LBA4505).

British Journal of Cancer (2011) 104, 1440–1451

www.bjcancer.com

Meta-analysis of immunohistochemical prognostic markers in resected pancreatic cancer

RA Smith[1], J Tang[1], C Tudur-Smith[1,2], JP Neoptolemos[1] and P Ghaneh[*,1]

[1]*Division of Surgery and Oncology, School of Cancer Studies, University of Liverpool, Royal Liverpool University Hospital, 5th Floor Duncan Building, Daulby Street, Liverpool L69 3GA, UK*

BACKGROUND: The potential prognostic value of several commonly investigated immunohistochemical markers in resected pancreatic cancer is variably reported. The objective of this study was to conduct a systematic review of literature evaluating p53, p16, smad4, bcl-2, bax, vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) expression as prognostic factors in resected pancreatic adenocarcinoma and to conduct a subsequent meta-analysis to quantify the overall prognostic effect.

METHODS: Relevant literature was identified using Medline, EMBASE and ISI Web of Science. The primary end point was overall survival assessed on univariate analysis. Only studies analysing resected pancreatic adenocarcinoma were eligible for inclusion and the summary $\log_e$ hazard ratio (logHR) and variance were pooled using an inverse variance approach. Evidence of heterogeneity was evaluated using the χ^2 test for heterogeneity and its impact on the meta-analysis was assessed by the I^2 statisic. Hazard ratios greater than one reflect adverse survival associated with positive immunostaining.

RESULTS: Vascular endothelial growth factor emerged as the most potentially informative prognostic marker (11 eligible studies, $n=767$, HR = 1.51 (95% confidence interval, CI = 1.18–1.92)) with no evidence of any significant publication bias (Egger's test, $P=0.269$). Bcl-2 (5 eligible studies, $n=314$, HR = 0.51 (95% CI = 0.38–0.68)), bax (5 studies, $n=274$, HR = 0.63 (95% CI = 0.48–0.83)) and p16 (3 studies, $n=229$, HR = 0.63 (95% CI = 0.43–0.92)) also returned significant overall survival differences, but in smaller patient series due to a lack of evaluable literature. Neither p53 (17 studies, $n=925$, HR = 1.22 (95% CI = 0.96–1.56)), smad4 (5 studies, $n=540$, HR = 0.88 (95% CI = 0.61–1.27)) nor EGFR (4 studies, $n=250$, HR = 1.35 (95% CI = 0.80–2.27)) was found to represent significant prognostic factors when analysing the pooled patient data. There was evidence of significant heterogeneity in four of the seven study groups.

CONCLUSION: These results support the case for immunohistochemical expression of VEGF representing a significant and reproducible marker of adverse prognosis in resected pancreatic cancer.

British Journal of Cancer (2011) **104,** 1440–1451. doi:10.1038/bjc.2011.110 www.bjcancer.com
Published online 29 March 2011

Keywords: immunohistochemistry; molecular; pancreatic cancer; meta-analysis; prognosis

Pancreatic ductal adenocarcinoma is characterised by its singularly aggressive tumour biology and unfavourable patient outcomes. Despite overall 5-year survival rates of <5%, previous randomised trials have demonstrated that for patients presenting with localised disease, resection with administration of adjuvant chemotherapy is associated with 5-year survival rates of over 20% (Neoptolemos *et al*, 2004; Oettle *et al*, 2007).

Reliable identification of molecular prognostic markers is important in order to facilitate the rational selection of potential therapeutic targets in the development of novel cancer therapies and to allow meaningful and reproducible risk stratification as part of clinical trials. There is marked disparity in the literature between individual studies as to the relative prognostic impact of several immunohistochemical tissue markers in pancreatic cancer. This may, in part, be explained by heterogeneity in patient selection due to inclusion of resected and unresected patients in survival analyses or inclusion of mixed tumour types and laboratory methodology when comparing different studies. The objective of the present study was to conduct a systematic review and meta-analysis of published literature investigating the commonly reported immunohistochemical prognostic markers in resected primary tumour material from patients with pancreatic adenocarcinoma and to identify potential sources of heterogeneity when comparing the results of individual studies.

*Correspondence: Dr P Ghaneh; E-mail: p.ghaneh@liverpool.ac.uk
[2] Current address: North West Hub for Trial Methodology Research, Centre for Medical Statistics and Health Evaluation, University of Liverpool, Liverpool, UK
Received 10 December 2010; revised 2 March 2011; accepted 8 March 2011; published online 29 March 2011

MATERIALS AND METHODS

Search strategy

Medline, EMBASE and ISI Web of Science were searched to identify potentially relevant published literature. No chronological search criteria were applied. Existing systematic reviews and reference lists were also checked for any potentially relevant

additional studies. The most widely investigated and biologically relevant immunohistochemical tissue markers for pancreatic cancer were selected for meta-analysis. These comprised p53, smad4, p16, bcl-2, bax, vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR).

Selection criteria

The following criteria were used to search English language articles and abstracts: '(*marker*)' AND ('pancreas' OR 'pancreatic') AND ('survival' OR 'prognosis' OR 'prognostic'). Each search was repeated for individual markers by substituting the name of marker of interest along with relevant synonyms: 'p53' OR 'TP53'; 'p16' OR 'p16*' OR 'CDKN2A'; 'smad4' OR 'smad-4' OR 'smad*'; 'DPC4' OR 'DPC-4' OR 'DPC*'; 'bcl-2' OR 'bcl2' OR 'bcl' OR 'bcl*'; 'bax'; 'vascular endothelial growth factor' OR 'VEGF' OR 'VEGF*'; 'epidermal growth factor receptor' OR 'EGFR' OR 'c-erbB*' OR 'erbB*' OR 'HER*'. The search was performed in November 2009. Abstracts were initially checked for relevance and the full article was retrieved for all potentially eligible studies. Where part or all of the same patient series was included in more than one publication, only the more recent or most complete study was included in the analysis in order to avoid duplication of the same survival data.

The following inclusion criteria were used to select literature: only cases of resected pancreatic adenocarcinoma analysed, immunohistochemical expression assessed in resected primary tumour material, dichotomised univariate survival analysis reported (i.e. positive *vs* negative staining) and overall survival times used in analysis. For the analysis of VEGF, only studies investigating the prognostic value of VEGF-A expression were included. Authors were contacted for unpublished results in cases where insufficient survival data were reported to estimate the $\log_e$ hazard ratio (logHR) and variance. Due to the minority of studies reporting multivariable analyses, no attempt was made to use any adjusted survival data as part of this meta-analysis (i.e. only univariate survival data were extracted).

End points

The primary outcome measure was overall survival (i.e. date of resection to date of death). Additional details were also collected in order to identify potential sources of heterogeneity. These included the specific primary antibody (and dilution) used for immunohistochemistry, the scoring criteria used to define positive staining and relevant clinico-pathological data. An assessment of study methodology was made according to previously defined criteria (Hayden *et al*, 2006; McShane *et al*, 2006). These principles were used to define 20 individual study characteristics, which were deemed to be key factors to report in an immunohistochemical prognostic study (Table 1). For any criterion not fulfilled according to the information outlined in the article, one point was deducted from a maximum of 20 and the final score was recorded as a percentage. The eligibility criteria and quality scoring were assessed by two independent investigators. Any disagreement was resolved by discussion.

Statistical analysis

Previously reported indirect methods were utilised for extracting the logHR and variance due to the paucity of prognostic literature, which report these values directly (Parmar *et al*, 1998; Williamson *et al*, 2002; Tierney *et al*, 2007). These values were either calculated from the HR and 95% confidence interval (CI) where quoted, the log rank *P*-value, or from the Kaplan–Meier survival curves directly. The software used for these indirect calculations was designed by Matthew Sydes and Jayne Tierney of the Medical Research Council Clinical Trials Unit, London, UK

Table 1 Methodological scoring criteria used

Study group	
Study population adequately described	
Gender/age	1 Point
Histology	1 Point
Period of recruitment	1 Point
Inclusion/exclusion criteria used	1 Point
Study attrition	
>90% of cases identified included in final analysis	1 Point
Reasons for attrition/loss to follow-up given	1 Point
Peri-operative mortality details	1 Point
Scientific methodology	
IHC methodology outlined	
Details of 1°/2° Abs used	1 Point
Concentration of 1° Abs used	1 Point
Positive/negative controls outlined	1 Point
Description of scoring technique	
>1 independent scorer	1 Point
Scorers blinded to clinical data	1 Point
Criteria for positivity clearly outlined	
Distribution (cytoplasm *vs* membranous *vs* nuclear)	1 Point
% positive cells for immunostaining classification	1 Point
Confounding factors considered	
Adjuvant therapy details provided	1 Point
Histological breakdown according to IHC staining	1 Point
Statistical analysis	
HR (confidence interval) provided	1 Point
Exact *P*-value quoted	1 Point
Numbers at risk for Kaplan–Meier curves	1 Point
Number of censored cases recorded	1 Point

Abbreviations: HR = hazard ratio; IHC = immunohistochemical.

(Tierney *et al*, 2007). The logHR and variance for individual studies were entered into RevMan 4.2 (Cochrane collaboration, Oxford, UK) and pooled using a random effects inverse variance approach. The overall prognostic effect of positive immunostaining was recorded as an HR and 95% CI (i.e. an HR>1 reflecting adverse survival associated with positive immunostaining). Heterogeneity was assessed using a χ^2 test for heterogeneity with a *P*-value of <0.10 taken to reflect the presence of significant heterogeneity. The I^2 statistic was calculated to quantify the degree of heterogeneity (Higgins and Thompson, 2002). A *P*-value of <0.050 was taken to reflect significance for all other analyses. Publication bias was assessed by inspection of the funnel plot with Egger's regression. Continuous data were compared using Spearman's rank correlation and two-sided Mann–Whitney testing for categorical data.

RESULTS

VEGF

The initial search returned a total of 255 studies. Following review of these abstracts, 20 potentially relevant studies were identified as eligible of which nine were excluded for the following reasons: duplicated series of patients (Ikeda *et al*, 1999; Niedergethmann *et al*, 2000; Tang *et al*, 2001), only VEGF-C and/or VEGF-D analysed (Kurahara *et al*, 2004; Zhang *et al*, 2007), no dichotomised univariate survival analysis reported (Ellis *et al*, 1998; Fujioka *et al*, 2001), mix of resected and unresected cases included in survival analysis (Chung *et al*, 2006) and only VEGF receptor status analysed (Büchler *et al*, 2002).

The 11 eligible studies (all retrospective) included a total of 767 patients with a median number of 62 patients per study (range = 19–142). Table 2 outlines the demographic,

Table 2 Methodological and clinico-pathological data for eligible prognostic studies evaluating VEGF, bcl-2, bax and p16

Reference	*n*	HR (95% CI)	Signi-ficant	I° Ab (+dilution)	IHC +ve	IHC cutoff (%)	Male	Age	NI	T3/T4	Well	Mod.	Poor	Adjuvant therapy
VEGF														
Itakura *et al* (1997)	75	1.12 (0.69–1.82)	No	NC (30 $\mu g\,ml^{-1}$)	48 (64)	>10	46 (61)	62	47 (63)	43 (57)	13 (17)	44 (59)	18 (24)	NS
Fujimoto *et al* (1998)	50	0.78 (0.44–1.40)	No	Santa Cruz A20 (1:200)	28 (40)	NS	28 (56)	62	29 (58)	34 (68)	9 (18)	31 (62)	10 (20)	NS
Seo *et al* (2000)	142	1.46 (1.02–2.09)	Yes	Santa Cruz (NS)	94 (66)	>30	79 (56)	64	95 (67)	NS	NS	NS	NS	NS
Ikeda *et al* (2001)	48	2.74 (1.44–5.20)	Yes	Santa Cruz (1:200)	31 (65)	>10	37 (77)	64	24 (50)	40 (83)	15 (31)	28 (58)	5 (11)	48 (100)
Knoll *et al* (2001)	19	2.37 (0.88–6.40)	No	R&D Ab293NA (1:200)	13 (68)	>5	11 (58)	58	18 (95)	1 (5)	1 (5)	12 (63)	6 (32)	0 (0)
Niedergethmann *et al* (2002)	70	2.48 (1.22–5.05)	Yes	Santa Cruz (1:200)	28 (40)	>10	42 (60)	63	41 (59)	NS	25 (36)	45 (64)	22 (31)	
Kuwahara *et al* (2003)	55	2.08 (1.12–3.88)	Yes	Santa Cruz sc152 (1:200)	39 (71)	>50	34 (62)	64	30 (55)	40 (73)	13 (24)	33 (60)	9 (16)	NS
Lim *et al* (2004)	72	0.82 (0.49–1.37)	No	Santa Cruz (1:2000)	23 (32)	>10	43 (60)	60	38 (53)	59 (82)	14 (19)	44 (61)	14 (19)	26 (36)
Khorana *et al* (2005)	124	1.30 (0.87–1.95)	No	Zymed (1:50)	70 (56)	>5	69 (56)	67	56 (45)	69 (58)	23 (19)	52 (43)	45 (38)	88 (79)
Tang *et al* (2006)	50	1.46 (0.84–2.54)	No	NS (2 $\mu g\,ml^{-1}$)	25 (50)	>10	25 (50)	63	39 (78)	25 (50)	15 (30)	31 (62)	4 (8)	NS
Ai *et al* (2008)	62	2.34 (1.41–3.89)	Yes	Neomarkers (NS)	37 (60)	>10	36 (58)	65	49 (79)	32 (52)	17 (27)	15 (24)	30 (48)	0 (0)
bcl-2														
Bold *et al* (1999)	70	0.64 (0.35–1.18)	No	DAKO (NS)	23 (33)	>25%	36 (51)	64	32 (46)	NS	15 (22)	37 (55)	15 (22)	19 (27)
Nio *et al* (2001b)	66	0.45 (0.25–0.82)	Yes	DAKO M0887 (1:100)	16 (24)	>5%	31 (47)	66	54 (82)	NS	33 (50)	29 (44)	4 (6)	36 (55)
Magistrelli *et al* (2006)	67	0.56 (0.33–0.96)	Yes	DAKO c124 (1:40)	45 (67)	>5%	45 (67)	63	34 (51)	40 (62)	14 (21)	28 (42)	15 (22)	30 (45)
Sarela *et al* (2002)	52	0.50 (0.08–3.33)	No	DAKO (1:40)	6 (12)	>10%	27 (52)	64	40 (78)	49 (94)	11 (22)	24 (47)	16 (31)	NS
Dong *et al* (2005b)	59	0.43 (0.25–0.74)	Yes	DAKO M124(1:100)	21 (36)	>5%	19 (32)	55	54 (82)	NS	19 (32)	21 (36)	19 (32)	NS
bax														
Friess *et al* (1998)	60	0.47 (0.23–0.97)	Yes	Santa Cruz (NS)	50 (83)	NS	32 (53)	63	38 (63)	NS	NS	NS	NS	NS
Evans *et al* (2001)	23	0.80 (0.28–2.29)	No	Santa Cruz (1:1600)	6 (26)	>5%	15 (65)	59	38 (63)	NS	5 (22)	13 (54)	5 (22)	0 (0)
Nio *et al* (2001b)	65	0.49 (0.28–0.85)	Yes	DAKO A3533 (1:100)	42 (65)	>10%	31 (47)	66	54 (82)	NS	33 (50)	29 (44)	4 (6)	36 (55)
Magistrelli *et al* (2006)	67	0.56 (0.33–0.95)	Yes	Zymed c2D2 (1:80)	36 (54)	>10%	45 (67)	63	34 (51)	40 (62)	14 (21)	28 (42)	15 (22)	30 (45)
Dong *et al* (2005b)	59	0.93 (0.57–1.52)	No	DAKO A3533 (1:100)	29 (49)	>10%	19 (32)	55	54 (82)	NS	19 (32)	21 (36)	19 (32)	NS
p16														
Naka *et al* (1998)	32	0.45 (0.21–0.96)	Yes	Santa Cruz C20 (1:500)	19 (59)	NS	20 (63)	65	23 (72)	13 (41)	NS	NS	NS	NS
Kawesha *et al* (2000)	157	0.82 (0.50–1.33)	No	Santa Cruz (1:100)	21 (13)	>5%	100 (64)	60	71 (46)	NS	21 (13)	77 (49)	59 (38)	13 (8)
Gerdes *et al* (2002)	40	0.51 (0.25–1.04)	No	Pharmingen G175–405 (1:50)	13 (33)	>5%	22 (55)	NS	16 (40)	NS	NS	NS	NS	0 (0)

Abbreviations: CI = confidence interval; HR = hazard ratio; IHC = immunohistochemical; NC = non-commercial; NS = not specified; VEGF = vascular endothelial growth factor. % in parentheses unless otherwise stated. IHC and/or clinico-pathological data were incompletely reported in some studies. Well/Mod/Poor refers to tumour differentiation.

clinico-pathological, methodological and outcome characteristics of these studies. The median quality score was recorded as 70% (range = 60–95%). There was no significant difference in median quality scores between significant and non-significant studies (Mann–Whitney, $P = 0.516$). Similarly, there was no significant correlation between study size and quality scores (Spearman's $\rho = 0.139$, $P = 0.698$). Figure 1 illustrates the Forrest plot for the survival data. Significant heterogeneity was demonstrated according to Cochran's χ^2 test ($\chi^2 = 22.08$, $P = 0.01$; $I^2 = 54.7\%$). The combined HR was recorded as 1.51 (95% CI = 1.18–1.92), indicating that positive immunostaining for VEGF was significantly associated with adverse survival in the pooled patient group. When assessing the funnel plot for this analysis (Figure 2), the data points approximated a symmetrical distribution (Egger's test, $P = 0.269$), indicating that publication bias is unlikely to be a significant confounding factor in describing this relationship.

The median proportion of patients classified as VEGF positive in the included studies was recorded as 60% (range = 32–71%). The proportion of VEGF positive cases reported in each study failed to exhibit any correlation with the assessment of methodological quality (Spearman's $P = 0.491$) or the % cutoff used to define positive immunostaining (Spearman's $P = 0.388$). Only six studies reported the proportion of patients who received any form of adjuvant therapy (Table 2) and administered treatment modalities included a mix of both chemotherapy and chemoradiation. No studies reported use of any neoadjuvant therapy and only a single study reported use of intra-operative radiotherapy (Ikeda *et al*, 2001). Of the five studies that reported positive VEGF expression as a significant adverse prognostic variable, only three conducted some form of multivariate analysis. These three analyses included a variety of disparate covariates alongside VEGF. However, each reported that VEGF expression retained statistical significance.

bcl-2

The initial search returned a total of 232 abstracts of which 16 potentially eligible articles were retrieved. A total of 11 were excluded for the following reasons: duplicated series of patients (Nio *et al*, 2001a), mix of resected and unresected cases included (Gansauge *et al*, 1998; Mäkinen *et al*, 1998; Ohshio *et al*, 1998; Hu *et al*, 1999), inclusion of ampullary tumours (Sinicrope *et al*, 1996), no dichotomised univariate survival analysis conducted (Evans *et al*, 2001; Stipa *et al*, 2002; Sun *et al*, 2002) and insufficient survival data reported for indirect estimation of logHR and variance (Friess *et al*, 1998; Campani *et al*, 2001).

The five eligible studies included a total of 314 patients with a median number of 63 patients per study (range = 52–70) (Table 2). The median quality score was recorded as 75% (range = 65–85%) and the median proportion of bcl-2 positive cases was 33% (range = 12–67%). Figure 2 illustrates the Forrest plot for the pooled survival data. There was no evidence of any significant heterogeneity ($\chi^2 = 1.19$, $P = 0.88$). The combined HR was recorded as 0.51 (95% CI = 0.38–0.68), indicating a significant association between positive bcl-2 immunostaining and more favourable survival in the pooled patient group. Despite the limited number of studies included, the funnel plot for this analysis failed to demonstrate any obvious asymmetry (Figure 3). Three studies reported use of either adjuvant chemotherapy or chemoradiation and a single study (Bold *et al*, 1999) also reported use of neoadjuvant chemoradiation in 43 out of the 70 patients analysed. Of the two studies rejected due to incomplete survival data (Friess *et al*, 1998; Campani *et al*, 2001), both failed to observe any significant prognostic effect associated with bcl-2 expression. Neither study reported the direction of the prognostic effect.

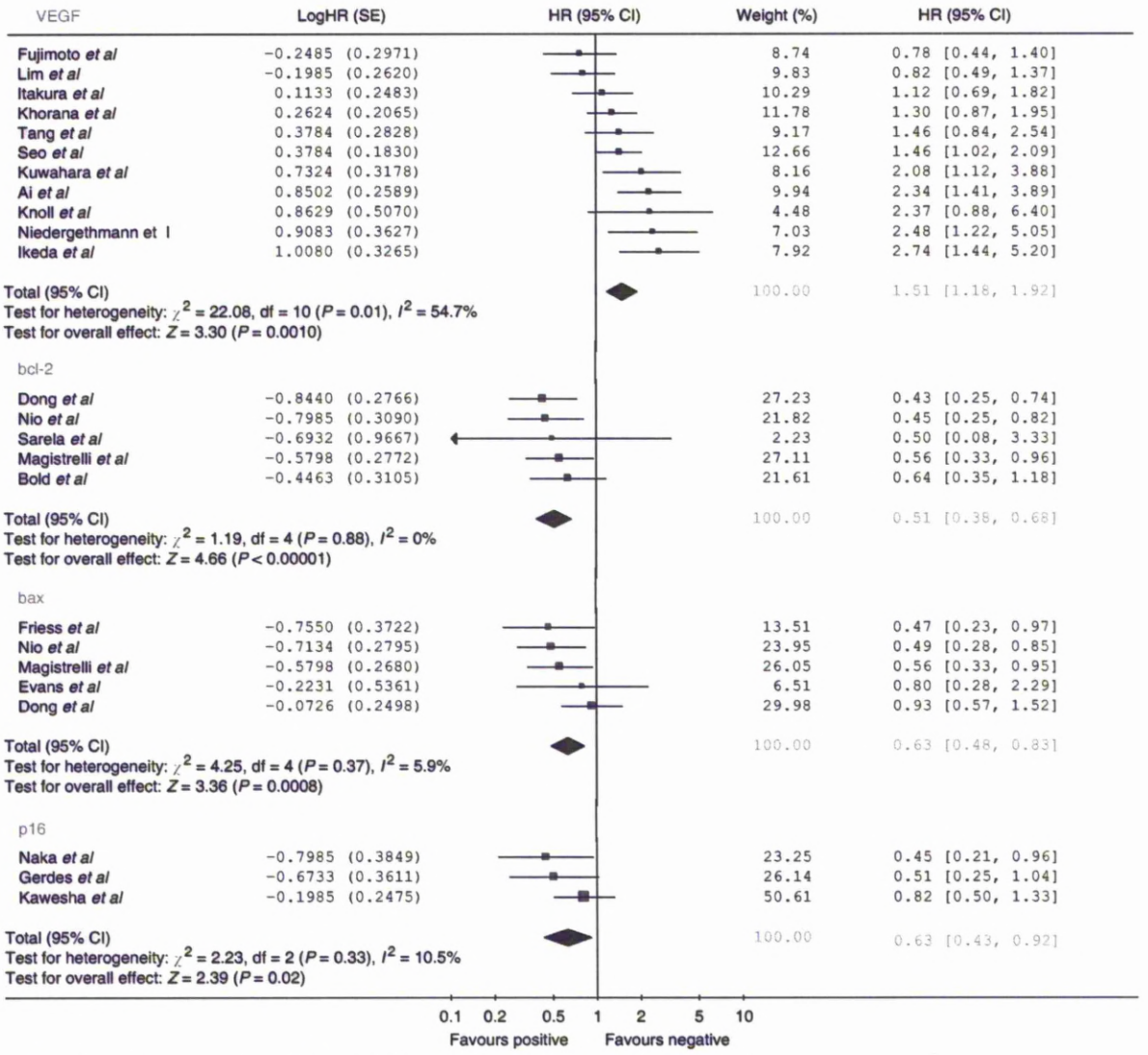


Figure 1 Forrest plot to assess overall effect of VEGF, bcl-2, bax and p16 expression on survival.

bax

The initial search yielded 76 studies. Following review of the abstracts, a total of seven potentially eligible articles were identified. Two of these were excluded due to either a duplicated patient series (Hashimoto *et al*, 2005) or the inclusion of periampullary cancers of non-pancreatic origin in the survival analysis (Tomazic *et al*, 2004). Three of the five eligible studies investigated the prognostic effect of both bcl-2 and bax and were, therefore, included in both meta-analyses (Magistrelli *et al*, 2006; Nio *et al*, 2001b; Dong *et al*, 2005b).

The five eligible studies investigating bax included a total of 274 patients with a median number of 60 patients per study (range = 23–67) (Table 2). The median quality score was 65% (range = 55–85%) and the median proportion of bax positive cases was 54% (range = 26–83%). Figure 2 illustrates the Forrest plot for the pooled survival data. There was no evidence of any significant heterogeneity ($\chi^2 = 4.25$, $P = 0.37$; $I^2 = 5.9\%$). The combined HR was recorded as 0.63 (95% CI = 0.48–0.83) and the funnel plot for this analysis is shown in Figure 3.

p16

The initial search returned 91 studies, seven of which were potentially relevant. Following review of these seven articles, three fulfilled all of the eligibility criteria. The remaining studies were rejected due to the inclusion of unresected cases (Hu *et al*, 1997; Biankin *et al*, 2002), no IHC used in tissue analysis (Ohtsubo *et al*, 2003) or only disease-free survival times reported (Jeong *et al*, 2005). A total of 229 patients were included in the pooled analysis. There was no evidence of any significant heterogeneity across the three included studies ($\chi^2 = 2.23$, $P = 0.33$; $I^2 = 10.5\%$). A combined HR of 0.63 (95% CI = 0.43–0.92) was obtained, indicating a significant association between p16 expression and more favourable survival.

p53

The initial search returned a total of 337 studies. Following review of these abstracts, 58 potentially relevant studies were retrieved of which 17 fulfilled all of the inclusion criteria. The remaining

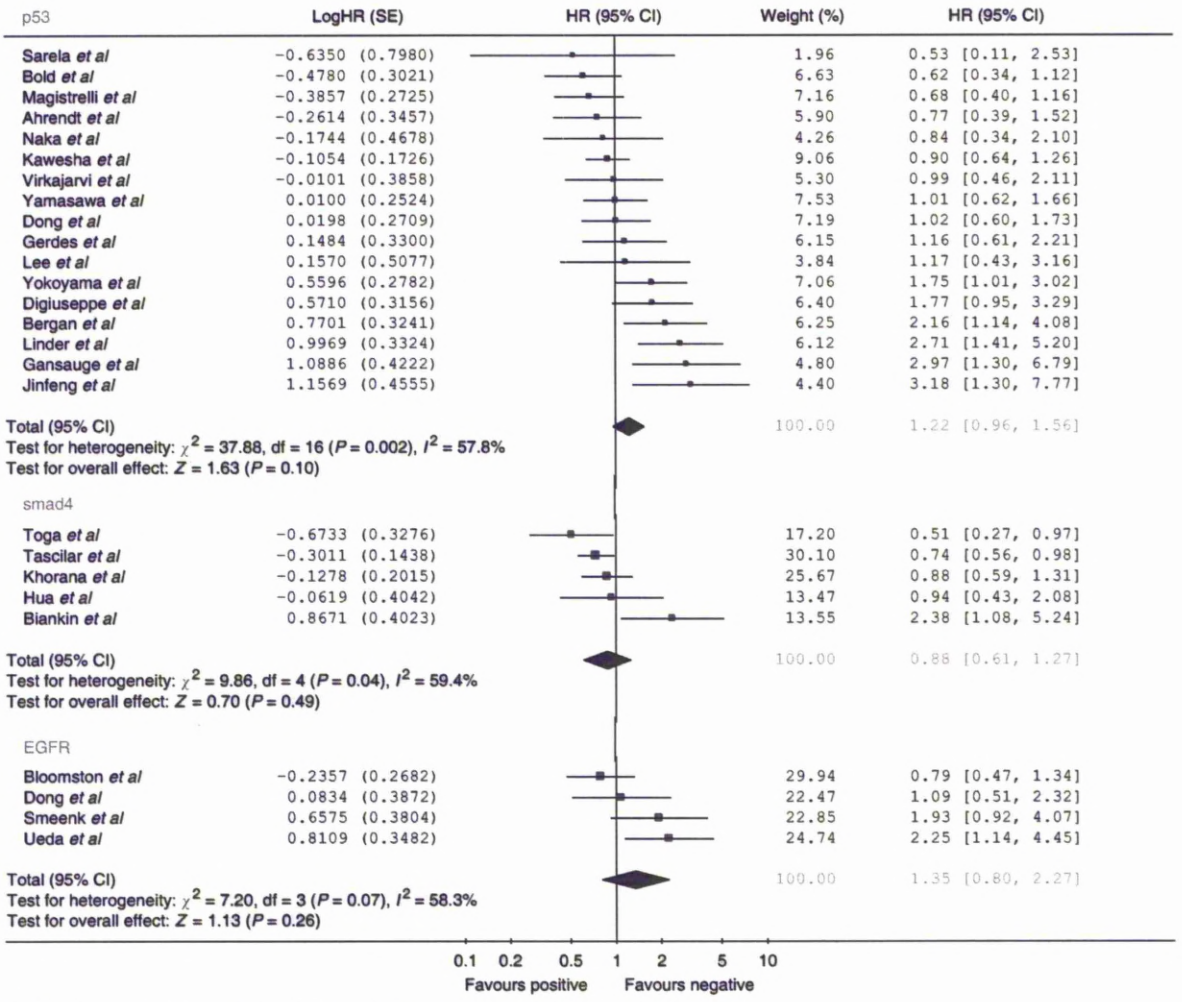


Figure 2 Forrest plot to assess overall effect of p53, smad4 and EGFR expression on survival.

studies were rejected for the following reasons: duplicated series of patients (Dergham *et al*, 1997a; Dong *et al*, 1998b; Nio *et al*, 1998; Nio *et al*, 1999; Dong *et al*, 2000; Linder *et al*, 2001; Nio *et al*, 2001a), no dichotomised univariate survival analysis conducted (Sessa *et al*, 1998; Karademir *et al*, 2000; Evans *et al*, 2001; Fujioka *et al*, 2001; Gazzaniga *et al*, 2001; Biankin *et al*, 2002; Dang *et al*, 2002; Hashimoto *et al*, 2005; Dong *et al*, 2007; Smeenk *et al*, 2007), no IHC used in tissue analysis (Weyrer *et al*, 1996; Li *et al*, 1999; Yamaguchi *et al*, 2000; Ohshio *et al*, 2002; Dong *et al*, 2003), unresected cases included in survival analysis (Zhang *et al*, 1994; Aizawa *et al*, 1996; Lundin *et al*, 1996; Coppola *et al*, 1998; Dergham *et al*, 1998; Mäkinen *et al*, 1998; Ohshio *et al*, 1998; Hu *et al*, 1999; Takikita *et al*, 2009), mix of different tumour types included (Sinicrope *et al*, 1996; Sato *et al*, 1997; Gansauge *et al*, 1998; Yu *et al*, 2004), only disease-free survival reported (Jeong *et al*, 2005) and insufficient survival data reported (Dergham *et al*, 1997b; Campani *et al*, 1999; Stipa *et al*, 2002; Hermanova *et al*, 2009).

The 17 eligible studies included a total of 925 patients with a median number of 48 patients per study (range = 26–157) (Table 3). Nuclear staining of p53 was used for scoring in all cases. Five studies (29%) reported a significant adverse association between p53 expression and survival. The median quality score was recorded as 65% (range = 45–90%) and the median proportion of patients exhibiting positive p53 immunostaining was 47% (range = 25–68%). There was no significant association between the IHC cutoff score used and the proportion of cases classified as p53 positive (Spearman's $\hat{n} = 0.389$, $P = 0.206$). Furthermore, there was no significant difference in median quality scores between significant and non-significant studies (Mann–Whitney, $P = 0.243$).

Figure 2 illustrates the Forrest plot for the survival data. There was no evidence of any significant publication bias (Egger's test, $P = 0.298$). Significant heterogeneity was demonstrated according to Cochran's χ^2 test ($\chi^2 = 37.88$, $P = 0.002$; $I^2 = 57.8\%$). The combined HR was recorded as 1.22 (95% CI = 0.96–1.56), indicating no significant overall association between p53 expression and survival. Of the four studies excluded due to incomplete reporting of survival data, only one reported a significant association between p53 expression and survival (Stipa *et al*, 2002).

smad4

The initial search returned 81 studies. Following review of these abstracts, five potentially relevant studies were identified, which were all found to be eligible for analysis. The combined number of patients was 540 with a median of 88 patients per study (range = 34–249) (Table 3). The median quality score was 75%

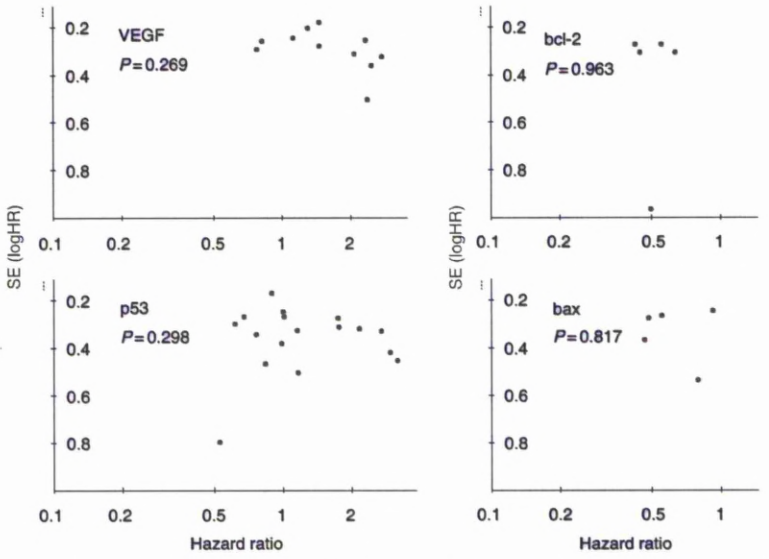


Figure 3 Funnel plots to assess publication bias for VEGF, bcl-2, bax and p53 meta-analyses. *Note*: *P*-values for result of Egger's regression to assess publication bias.

Table 3 Methodological and clinico-pathological data for eligible prognostic studies evaluating p53, smad4 and EGFR

Reference	*n*	HR (95% CI)	Signi-ficant	I° Ab (+ dilution)	IHC +ve	IHC cutoff (%)	Male	Age	NI	T3/T4	Well	Mod.	Poor	Adjuvant therapy
p53														
DiGiuseppe *et al* (1994)	48	1.77 (0.95–3.29)	No	Novocastra CM-1 (1:1000)	26 (54)	NS	25 (52)	61	NS	NS	NS	NS	NS	NS
Yokoyama *et al* (1994)	57	1.75 (1.01–3.02)	Yes	Novocastra DO7 (1:100)	33 (58)	NS	NS	64	25 (45)	27 (47)	37 (65)	20 (35)	NS	
Lee *et al* (1995)	26	1.17 (0.43–3.16)	No	Biogenex CM1 (NS)	7 (27)	NS	14 (54)	NS	NS	NS	2 (8)	20 (77)	4 (15)	NS
Linder *et al* (1997)	48	2.71 (1.41–5.20)	Yes	DAKO DO7 (1:50)	22 (46)	>1	36 (68)	66	18 (38)	26 (49)	5 (9)	18 (34)	30 (57)	NS
Virkajärvi *et al* (1997)	36	0.99 (0.46–2.11)	No	Novocastra CM-1 (1:1000)	15 (42)	>1	16 (44)	64	NS	NS	NS	NS	NS	NS
Naka *et al* (1998)	32	0.84 (0.34–2.10)	No	Novocastra BP53-12 (1:50)	19 (59)	NS	20 (63)	65	23 (72)	13 (41)	NS	NS	NS	NS
Bold *et al* (1999)	70	0.62 (0.34–1.12)	No	Oncogene DO1 (NS)	33 (47)	>25	36 (51)	64	38 (54)	NS	15 (22)	37 (56)	15 (22)	19 (27)
Gansauge *et al* (1999)	26	2.97 (1.30–6.79)	Yes	Oncogene DO1 (1:500)	11 (42)	NS	12 (50)	59	22 (85)	NS	NS	NS	NS	26 (100)
Ahrendt *et al* (2000)	43	0.77 (0.39–1.52)	No	DAKO DO7 (1:2000)	26 (60)	>33	24 (55)	63	23 (53)	22 (51)	11 (26)	23 (55)	8 (19)	29 (66)
Bergan *et al* (2000)	60	2.16 (1.14–4.08)	Yes	Novocastra DO7 (1:100)	15 (25)	>5	41 (50)	62	18 (30)	21 (35)	25 (42)	23 (38)	12 (20)	0 (0)
Kawesha *et al* (2000)	157	0.90 (0.64–1.26)	No	DAKO DO7 (1:300)	64 (41)	>5	100 (64)	60	71 (46)	NS	21 (13)	77 (49)	59 (38)	13 (8)
Gerdes *et al* (2002)	40	1.16 (0.61–2.21)	No	DAKO DO7 (1:400)	13 (33)	>10	22 (55)	NS	16 (40)	NS	NS	NS	NS	0 (0)
Sarela *et al* (2002)	52	0.53 (0.11–2.53)	No	DAKO DO7 (1:100)	28 (54)	>10	27 (52)	64	40 (78)	49 (94)	11 (22)	24 (47)	16 (31)	NS
Yamasawa *et al* (2002)	72	1.01 (0.62–1.66)	No	Oncogene DO1 (2 μg/ml)	34 (47)	>20	34 (47)	65	21 (29)	42 (58)	35 (49)	32 (44)	5 (7)	41 (57)
Dong *et al* (2005a)	59	1.02 (0.60–1.73)	No	DAKO DO7 (1:20)	40 (68)	>10	38 (64)	NS	47 (80)	NS	19 (32)	21 (36)	19 (32)	NS
Magistrelli *et al* (2006)	67	0.68 (0.40–1.16)	No	DAKO DO7 (1:50)	32 (48)	>5	45 (67)	63	34 (51)	40 (62)	14 (21)	28 (42)	15 (22)	30 (45)
Jinfeng *et al* (2007)	32	3.18 (1.30–7.77)	Yes	DAKO DO7 (1:50)	13 (41)	>10	19 (59)	63	18 (56)	23 (72)	11 (34)	18 (56)	3 (10)	NS
smad4														
Tascilar *et al* (2001)	249	0.74 (0.56–0.98)	Yes	Santa Cruz B8 (1:100)	111 (46)	NS	139 (56)	65	NS	NS	NS	NS	NS	NS
Biankin *et al* (2002)	45	2.38 (1.08–5.24)	Yes	Santa Cruz B8 (NS)	10 (22)	>5	27 (60)	61	21 (47)	NS	5 (11)	28 (62)	12 (27)	8 (16)
Hua *et al* (2003)	34	0.94 (0.43–2.08)	No	Santa Cruz B8 (1:100)	26 (76)	NS	22 (65)	55	14 (41)	NS	27 (79)	7 (21)	NS	
Toga *et al* (2004)	88	0.51 (0.27–0.97)	Yes	Santa Cruz B8 (1:100)	13 (15)	>10	43 (49)	66	78 (89)	33 (37)	37 (42)	45 (51)	6 (7)	58 (66)
Khorana *et al* (2005)	124	0.88 (0.59–1.31)	No	Santa Cruz (1:400)	59 (48)	>5	69 (56)	67	56 (45)	69 (58)	23 (19)	52 (43)	45 (38)	88 (79)
EGFR														
Dong *et al* (1998a)	57	1.09 (0.51–2.32)	No	Oncogene 985/996 (1:20)	39 (68)	NS	20 (35)	55	46 (81)	NS	18 (32)	22 (39)	17 (30)	7 (12)
Ueda *et al* (2004)	76	2.25 (1.14–4.45)	Yes	Zymed 31G7 (1:200)	47 (62)	>10	57 (75)	63	59 (78)	NS	11 (14)	32 (42)	33 (43)	NS
Bloomston *et al* (2006)	71	0.79 (0.47–1.34)	No	Dakocytomation 218C9 (NS)	49 (69)	>1	40 (56)	65	41 (58)	57 (81)	6 (9)	45 (63)	20 (28)	NS
Smeenk *et al* (2007)	46	1.93 (0.92–4.07)	No	DAKO H11 (NS)	11 (24)	>1	37 (66)	63	29 (52)	34 (61)	6 (11)	43 (77)	7 (12)	19 (34)

Abbreviations: CI = confidence interval; EGFR = epidermal growth factor receptor; HR = hazard ratio; IHC = immunohistochemical; NC = non-commercial; NS = not specified. % in parentheses unless otherwise stated. IHC and/or clinico-pathological data were incompletely reported in some studies. Well/Mod./Poor refers to tumour differentiation.

(range = 60–95%) and the median proportion of patients exhibiting positive smad4 immunostaining was 45% (range = 15–76%). Figure 2 illustrates the Forrest plot. There was evidence of significant heterogeneity across the included studies ($\chi^2 = 9.86$, $P = 0.04$; $I^2 = 59.4\%$). A combined HR of 0.88 (95% CI = 0.61–1.27) was recorded, indicating no significant overall association between smad4 expression and survival in the pooled patient group.

EGFR

The initial search identified 324 studies. Following review of these abstracts, 10 potentially relevant articles were retrieved. Six of these studies were rejected for the following reasons: duplicated series of patients (Uegaki *et al*, 1997; Ueda *et al*, 2006), no dichotomised univariate survival analysis conducted (Yamanaka *et al*, 1993; Zhang and Yuan, 2002) and unresected cases included in analysis (Gansauge *et al*, 1998; Takikita *et al*, 2009). The four eligible studies included a total of 250 patients (Table 3). Only a single study reported a significant relationship between EGFR expression and survival (Ueda *et al*, 2004). The median quality score was 70% (range = 65–70%). Figure 2 illustrates the Forrest plot for the pooled data. Significant heterogeneity was demonstrated ($\chi^2 = 7.20$, $P = 0.07$). The combined HR was recorded as 1.35 (95% CI = 0.80–2.27), indicating no significant overall association between EGFR expression and survival.

DISCUSSION

Previous meta-analyses of studies investigating the prognostic value of molecular markers have been published for different malignancies. These include VEGF (Delmotte *et al*, 2002; Kyzas *et al*, 2005a; Des Guetz *et al*, 2006), bcl-2 (Martin *et al*, 2003; Callagy *et al*, 2008) and p53 (Kyzas *et al*, 2005b; Malats *et al*, 2005). To date, no such meta-analysis has been undertaken for any studies evaluating immunohistochemical prognostic markers in resected pancreatic cancer.

Meta-analysis of prognostic literature is associated with a number of inherent limitations. One of these key limitations is the general prevalence of retrospective study design in this setting. None of the studies included in the current meta-analysis specified a prospective design and archived paraffin-embedded tumour material was utilised for IHC in all cases. This indicates that availability of tissue is invariably the main determinant of study size rather than any specific considerations relating to adequate statistical power in order to reliably detect a prognostic effect for the marker of interest. The availability and adequacy of corresponding clinico-pathological data is also a significant consideration in retrospective studies of this type and we identified several studies reporting incomplete datasets with regard to histopathological details. Alongside this, an additional hindrance to meta-analysis of prognostic literature is the general lack of multivariable survival data. This is usually attributable to the fact that the number of patients included in each study is typically small, precluding any meaningful attempt at analysing multiple covariates.

Additional challenges in the interpretation and comparison of immunohistochemical prognostic studies include variability in patient selection (i.e. resected and unresected cases, inclusion of non-pancreatic periampullary tumours), disparate immunohistochemical criteria used for prognostic classification, bias associated with the statistical approach to analysis of survival data (e.g. selection of data-driven cutoff values for continuous variables), incomplete reporting of survival data, duplicated patient series and publication bias arising as a result of selective reporting of 'positive' studies (Altman, 2001). In order to overcome some of these comparative difficulties, specific inclusion criteria were applied in order to select studies for meta-analysis. Only studies including resected pancreatic adenocarcinoma were included in order to avoid any confounding effects on survival associated with differing proportions of resected and unresected cases. Any studies including periampullary tumours of non-pancreatic origin were also excluded due to the disparity in survival outcomes characteristically associated with ampullary, duodenal and bile duct adenocarcinomas when compared with pancreatic adenocarcinoma (Riall *et al*, 2006). Furthermore, in cases where part or all of the same patient series was included in more than one publication, only the more recent or most complete study was included in the analysis in order to avoid duplicating the same patient data for the immunohistochemical marker of interest. For those studies where insufficient survival data was reported to generate indirect calculations for the logHR and variance, authors were contacted for additional survival data. However, in all cases the authors were either unable to provide any supplementary data or no response was received. The only supplementary raw data obtained was for two studies previously conducted at our own institution (Kawesha *et al*, 2000; Evans *et al*, 2001). Therefore, no subsequent attempt to request individual patient survival data for all eligible studies was undertaken, although this would have been potentially beneficial.

When analysing the overall relationships between individual study size, reported prognostic significance and methodological quality scores in the present study, there was a significant trend towards superior methodological quality in larger studies as one might reasonably expect, despite the fact that study size itself was not one of the criteria used for quality scoring. When considering the overall effect of potential publication bias in this analysis, only a minority of studies (21 out of 50) actually reported a statistically significant prognostic result. Furthermore, the funnel plots and Egger's tests for the individual analyses, although more difficult to interpret when fewer studies were included, were not generally indicative of any strong publication bias.

Vascular endothelial growth factor emerged as the most potentially informative immunohistochemical prognostic marker from the pooled data. Vascular endothelial growth factor comprises four ligands (VEGF-A, VEGF-B, VEGF-C and VEGF-D), which exhibit specific binding profiles with three transmembrane VEGF receptors (VEGF-I, -II and -III) and promote intracellular tyrosine kinase cascades when activated. The VEGF-A (usually referred to simply as VEGF) mediates the key pro-angiogenic properties of proliferation and migration of endothelial cells along with increasing vascular permeability (Yamazaki and Morita, 2006; Dallas *et al*, 2007). Alternate gene splicing results in a number of VEGF-A isoforms of differing amino-acid lengths, the smaller of which (e.g. 121 and 165) are secreted while the larger (e.g. 189 and 206) remains cell associated. VEGF-C and VEGF-D are implicated in the process of lymphangiogenesis (Achen and Stacker, 2008) while the function of VEGF-B is incompletely understood (Nash *et al*, 2006). Pancreatic cancer cells have been demonstrated to express both VEGF ligand and its receptors, implicating a potential VEGF-mediated autocrine loop in the proliferation of pancreatic malignancy (Büchler *et al*, 2002).

The results from the present study demonstrate that, despite variability between eligible studies as to the relative prognostic impact of VEGF expression in resected pancreatic adenocarcinoma, the observed survival trend is concordant with that reported for other malignancies in similar meta-analyses (Delmotte *et al*, 2002; Kyzas *et al*, 2005a, b; Des Guetz *et al*, 2006). When comparing the value for the pooled HR identified in the present study (1.51 (95% CI = 1.18–1.92)) with the above referenced studies, the order of magnitude for this effect is also broadly comparable for that quoted for both lung cancer (1.48 (95% CI = 1.27–1.72)) and colorectal cancer (1.65 (95% CI = 1.27–2.14)).

Significant heterogeneity was observed when analysing the logHR estimates from the eligible studies. Evaluation of the relevant methodological and clinico-pathological characteristics of each study revealed a number of potential sources of heterogeneity in study methodology. Nine studies reported use of commercially available anti-VEGF primary antibodies, all of which exhibit broadly comparable binding characteristics with the common splice variants of VEGF-A. When analysing the concentrations of primary antibody utilised, most studies reported comparable dilution ratios. However, the concentration was not specified in two studies. This issue is potentially relevant for the study reporting use of the lowest primary antibody dilution

(Lim *et al*, 2004) as this was one of only two studies, which indicated a contradictory prognostic effect when compared with the overall group (i.e. a non-significant trend towards adverse survival with negative VEGF immunostaining).

When reviewing the immunohistochemical criteria used for VEGF scoring, the majority of studies reported a scoring system based on cytoplasmic staining of tumour cells. Where the distribution of immunostaining used for scoring was not explicitly stated in the text (i.e. cytoplasmic, membranous, nuclear, stromal, etc.), the figures of representative VEGF staining presented in the relevant studies were all strongly indicative of cytoplasmic staining being used to define positive VEGF expression in cancer cells. All studies with one exception utilised a system of dichotomising patients according to the percentage of positively stained cells present. Despite the range of values used to define VEGF positivity across the included studies, there was no evidence of any significant association between the % cutoff value used and the corresponding proportion of VEGF positive patients reported. Furthermore, if including only the six studies, which used a standardised cutoff value of $>10\%$ for meta-analysis, the significance of the association between VEGF staining and adverse survival was unchanged (HR = 1.62 (95% CI = 1.09–2.40)—random effects). These observations indicate that differences in the specific scoring criteria used for immunohistochemical classification appear unlikely to have a significant confounding effect in describing the underlying relationship between VEGF expression and survival observed for the overall group.

Broadly comparable demographic and histological tumour characteristics were observed across the eligible VEGF studies, indicating that similar patient populations were evaluated in the combined analysis. Data relating to adjuvant therapy was only reported in 6 out of 11 studies and the treatment modalities included a mix of both chemotherapy and chemoradiation. Importantly, no studies reported any policy of selection of patients for adjuvant therapy based on VEGF tumour expression as immunohistochemical evaluation was undertaken on a retrospective basis in all cases. This was equally true for studies evaluating the other markers of interest.

Both bcl-2 and bax emerged as potentially relevant immunohistochemical prognostic factors. These proteins belong to the bcl-2 family and regulate apoptosis by mediating cytosolic release of cytochrome C from mitochondria in response to cellular stress. Cytochrome C binds to APAF-1 and cleaves caspase-9 into its active form, thereby initiating the activation of executioner caspases resulting in cytoskeletal degradation and cell death (Hamacher *et al*, 2008). The bcl-2-associated X protein (bax) promotes release of cytochrome C and consequently exhibits pro-apoptotic properties. In contrast, bcl-2 inhibits mitochondrial release of cytochrome C and has anti-apoptotic effects as a result. The finding that bax expression is associated with more favourable survival in resected pancreatic cancer is, therefore, concordant with its physiological role. The observation that the same relationship is consistently seen for bcl-2 expression appears paradoxical. However, this finding is mirrored in other malignancies (Martin *et al*, 2003; Callagy *et al*, 2008) and it is believed that a complex interaction of competitive dimerisations between pro- and anti-apoptotic proteins governs the cell's fate in response to apoptotic stimuli (Westphal and Kalthoff, 2003). It is difficult to draw any reliable conclusions from the current meta-analysis of bcl-2 and bax for the pancreatic literature due to the limited number of evaluable studies. However, the overall trend towards both bax and bcl-2 expression being associated with more favourable survival outcomes is generally consistent with the findings seen in other malignancies.

The tumour suppressor gene p16 (CDKN2A) has a key role in pancreatic carcinogenesis (Schutte *et al*, 1997). p16 is a cell-cycle checkpoint protein, which binds to cyclin-dependent kinases resulting in cell-cycle arrest at the G1/S checkpoint. The observation that positive immunostaining for p16 appears to represent a favourable prognostic feature is, therefore, also consistent with its tumour suppressor function. However, the small number of eligible studies included in this analysis again precludes any meaningful conclusions regarding the reproducibility of p16 expression as a reliable marker of prognosis in resected pancreatic cancer.

Of the various factors evaluated in the present study, the tumour suppressor protein p53 was found to represent the most extensively investigated immunohistochemical prognostic marker. It also exhibited a significant degree of heterogeneity in the reported association between immunostaining and survival for individual studies. Although the overall trend was towards overexpression of p53 resulting in adverse survival for the pooled data, this did not reach significance and there is no obvious explanation for the contradictory results seen between the various studies. The majority of studies used either the monoclonal DO-7, DO-1 or polyclonal CM-1 primary antibodies, which all exhibit immunoreactivity with both wild-type and mutant forms of p53. Due to the increased stability of mutant p53, most of the nuclear immunostaining seen reflects the presence of the mutant rather than wild-type p53 protein. Despite the marked differences between studies in terms of the proportion of cases classified as p53 positive, reported primary antibody dilutions used and cutoff values selected for immunohistochemical scoring, there was no clear association between any of these factors and either the direction of the prognostic effect or the reported magnitude of the HR, which might potentially explain the disparity in survival trends. As a result of these findings, immunohistochemical overexpression of p53 cannot be recommended as a reliable or reproducible marker of prognosis in resected pancreatic cancer from the available evidence.

The smad4 (or DPC4) protein is a central component of the intracellular signalling pathway for transforming growth factor β (TGF-β), and loss of smad4 expression represents an important event in the progression of PanINs to invasive malignancy (Wilentz *et al*, 2000). The results from the analysis of the five studies evaluating smad4 expression again demonstrate unexplained heterogeneity in the reporting of the prognostic effect of this marker. Biankin *et al* reported an entirely contradictory survival trend to the other four studies with loss of smad4 expression being associated with significantly *improved* patient survival despite use of the same primary antibody and otherwise broadly comparable study methodology and patient groups. This survival trend appears at odds with the accepted tumour suppressor role of smad4 in mediating the inhibitory signalling associated with the TGF-β pathway. Despite the fact that the patient series reported by Biankin *et al* only accounts for 8% of all patients included in the combined analysis and 14% of the weighting allocated to the pooled survival data, the discrepancy in the results is such that sufficient heterogeneity is introduced to require a random effects approach resulting in a non-significant result for the overall analysis. These findings further underline the difficulties in making any reliable conclusions regarding the relative prognostic value of immunohistochemical markers when analysed in limited patient series.

Epidermal growth factor receptor is the cell surface receptor for a family of extracellular ligands, which include EGF and TGF-α and is coded for by the c-erbB1 proto-oncogene. Activation of EGFR stimulates intracellular tyrosine kinase phosphorylation with consequent activation of a number of signalling cascades including the MAPK (mitogen-activated protein kinase) and Akt (protein kinase) pathways, which promote cell proliferation (Ciardello and Tortora, 2008). The analysis of the four eligible studies included in the current meta-analysis again fails to make a strong case for tumoural overexpression of EGFR representing a reproducible prognostic marker. However, the laboratory methodologies reported in the four studies demonstrated more marked variability

(e.g., use of four different EGFR primary antibodies) when compared with some of the other analyses.

Despite the inherent limitations of meta-analysing prognostic literature, the findings from the present study suggest that VEGF represents the most consistently reproducible molecular marker with prognostic value in resected pancreatic adenocarcinoma. This result is concordant with existing meta-analyses, which implicate a similar prognostic role for VEGF expression in other malignancies and lend further weight to the assertion that angiogenesis is a key determinant in driving pancreatic cancer progression. For several of the other markers evaluated in this study, directly contradictory prognostic effects were commonly observed with significant variability in the proportions of positive immunostaining reported, despite often broadly comparable patient groups and study methodologies. These results provide further evidence to suggest that in order to make reliable conclusions regarding immunohistochemical prognostic factors and to identify the relevance with which these factors can be translated into clinical use (e.g., individualised patient selection for adjuvant therapy modalities), large collaborative studies collecting tissue as part of prospective multicentre trials, with standardised approaches to both laboratory and statistical methodology, represent the optimal strategy to achieve these goals in the future (e.g. Farrell *et al*, 2009; Manuyakorn *et al*, 2010).

ACKNOWLEDGEMENTS

This study was supported by Cancer Research UK.

REFERENCES

Achen MG, Stacker SA (2008) Molecular control of lymphatic metastasis. *Ann N Y Acad Sci* **1131:** 225–234

Ahrendt SA, Brown HM, Komorowski RA, Zhu YR, Wilson SD, Erickson BA, Ritch PS, Pitt HA, Demeure MJ (2000) p21WAF1 expression is associated with improved survival after adjuvant chemoradiation for pancreatic cancer. *Surgery* **128:** 520–530

Ai KX, Lu LY, Huang XY, Chen W, Zhang HZ (2008) Prognostic significance of S100A4 and vascular endothelial growth factor expression in pancreatic cancer. *World J Gastroenterol* **14:** 1931–1935

Aizawa S, Sasaki M, Wada R, Koyama M, Yagihashi S (1996) P53 protein expression in pancreatic tumors and its relationship to clinicopathological factors and prognosis. *J Surg Oncol* **62:** 279–283

Altman DG (2001) Systematic reviews of evaluations of prognostic variables. *BMJ* **323:** 224–228

Bergan A, Gladhaug IP, Schjolberg A, Bergan AB, Clausen OP (2000) p53 accumulation confers prognostic information in resectable adenocarcinomas with ductal but not with intestinal differentiation in the pancreatic head. *Int J Oncol* **17:** 921–926

Biankin AV, Morey AL, Lee CS, Kench JG, Biankin SA, Hook HC, Head DR, Hugh TB, Sutherland RL, Henshall SM (2002) DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. *J Clin Oncol* **20:** 4531–4542

Bloomston M, Bhardwaj A, Ellison EC, Frankel WL (2006) Epidermal growth factor receptor expression in pancreatic carcinoma using tissue microarray technique. *Dig Surg* **23:** 74–79

Bold RJ, Hess KR, Pearson AS, Grau AM, Sinicrope FA, Jennings M, McConkey DJ, Bucana CD, Cleary KR, Hallin PA, Chiao PJ, Abbruzzese JL, Evans DB (1999) Prognostic factors in resectable pancreatic cancer: p53 and bcl-2. *J Gastrointest Surg* **3:** 263–277

Büchler P, Reber HA, Büchler MW, Friess H, Hines OJ (2002) VEGF-RII influences the prognosis of pancreatic cancer. *Ann Surg* **236:** 738–749

Callagy GM, Webber MJ, Pharoah PD, Caldas C (2008) Meta-analysis confirms bcl-2 is an independent prognostic marker in breast cancer. *BMC Cancer* **8:** 153

Campani D, Boggi U, Cecchetti D, Esposito I, Ceccarelli F, D'Antonio L, De Negri F, Mosca F, Bevilacqua G, Fornaciari G (1999) p53 overexpression in lymph node metastases predicts clinical outcome in ductal pancreatic cancer. *Pancreas* **19:** 26–32

Campani D, Esposito I, Boggi U, Cecchetti D, Menicagli M, De Negri F, Colizzi L, Del Chiaro M, Mosca F, Fornaciari G, Bevilacqua G (2001) Bcl-2 expression in pancreas development and pancreatic cancer progression. *J Pathol* **194:** 444–450

Chung GG, Yoon HH, Zerkowski MP, Ghosh S, Thomas L, Harigopal M, Charette LA, Salem RR, Camp RL, Rimm DL, Burtness BA (2006) Vascular endothelial growth factor, FLT-1, and FLK-1 analysis in a pancreatic cancer tissue microarray. *Cancer* **106:** 1677–1684

Ciardello F, Tortora G (2008) EGFR antagonists in cancer treatment. *N Engl J Med* **358:** 1160–1174

Coppola D, Lu L, Fruehauf JP, Kyshtoobayeva A, Karl RC, Nicosia SV, Yeatman TJ (1998) Analysis of p53, p21WAF1, and TGF-beta1 in human ductal adenocarcinoma of the pancreas: TGF-beta1 protein expression predicts longer survival. *Am J Clin Pathol* **110:** 16–23

Dallas NA, Fan F, Gray MJ, Van Buren II G, Lim SJ, Xia L, Ellis LM (2007) Functional significance of vascular endothelial growth factor receptors on gastrointestinal cancer cells. *Cancer Metastasis Rev* **26:** 433–441

Dang CX, Han Y, Qin ZY, Wang YJ (2002) Clinical significance of expression of p21 and p53 proteins and proliferating cell nuclear antigen in pancreatic cancer. *Hepatobiliary Pancreat Dis Int* **1:** 302–305

Delmotte P, Martin B, Paesmans M, Berghmans T, Mascaux C, Meert AP, Steels E, Verdebout JM, Lafitte JJ, Sculier JP (2002) VEGF and survival of patients with lung cancer: a systematic literature review and meta-analysis. *Rev Mal Respir* **19:** 577–584

Dergham ST, Dugan MC, Joshi US, Chen YC, Du W, Smith DW, Arlauskas P, Crissman JD, Vaitkevicius VK, Sarkar FH (1997a) The clinical significance of p21(WAF1/CIP-1) and p53 expression in pancreatic adenocarcinoma. *Cancer* **80:** 372–381

Dergham ST, Dugan MC, Kucway R, Du W, Kamarauskiene DS, Vaitkevicius VK, Crissman JD, Sarkar FH (1997b) Prevalence and clinical significance of combined K-ras mutation and p53 aberration in pancreatic adenocarcinoma. *Int J Pancreatol* **21:** 127–143

Dergham ST, Dugan MC, Sarkar FH, Vaitkevicius VK (1998) Molecular alterations associated with improved survival in pancreatic cancer patients treated with radiation or chemotherapy. *J Hepatobiliary Pancreat Surg* **5:** 269–272

Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, Breau JL, Perret GY (2006) Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. *Br J Cancer* **94:** 1823–1832

DiGiuseppe JA, Hruban RH, Goodman SN, Polak M, van den Berg FM, Allison DC, Cameron JL, Offerhaus GJ (1994) Overexpression of p53 protein in adenocarcinoma of the pancreas. *Am J Clin Pathol* **101:** 684–688

Dong M, Dong Q, Zhang H, Zhou J, Tian Y, Dong Y (2007) Expression of Gadd45a and p53 proteins in human pancreatic cancer: potential effects on clinical outcomes. *J Surg Oncol* **95:** 332–336

Dong M, Ma G, Tu W, Guo KJ, Tian YL, Dong YT (2005a) Clinicopathological significance of p53 and mdm2 protein expression in human pancreatic cancer. *World J Gastroenterol* **11:** 2162–2165

Dong M, Nio Y, Guo KJ, Tamura K, Tian YL, Dong YT (1998a) Epidermal growth factor and its receptor as prognostic indicators in Chinese patients with pancreatic cancer. *Anticancer Res* **18:** 4613–4619

Dong M, Nio Y, Sato Y, Tamura K, Song MM, Tian YL, Dong YT (1998b) Comparative study of p53 expression in primary invasive ductal carcinoma of the pancreas between Chinese and Japanese. *Pancreas* **17:** 229–237

Dong M, Nio Y, Tamura K, Song MM, Guo KJ, Guo RX, Dong YT (2000) Ki-ras point mutation and p53 expression in human pancreatic cancer: a comparative study among Chinese, Japanese, and Western patients. *Cancer Epidemiol Biomarkers Prev* **9:** 279–284

Dong M, Nio Y, Yamasawa K, Toga T, Yue L, Harada T (2003) p53 alteration is not an independent prognostic indicator, but affects the efficacy of adjuvant chemotherapy in human pancreatic cancer. *J Surg Oncol* **82:** 111–120

Dong M, Zhou JP, Zhang H, Guo KJ, Tian YL, Dong YT (2005b) Clinicopathological significance of Bcl-2 and Bax protein expression in human pancreatic cancer. *World J Gastroenterol* **11:** 2744–2747

Ellis LM, Takahashi Y, Fenoglio CJ, Cleary KR, Bucana CD, Evans DB (1998) Vessel counts and vascular endothelial growth factor expression in pancreatic adenocarcinoma. *Eur J Cancer* **34:** 337–340

Evans JD, Cornford PA, Dodson A, Greenhalf W, Foster CS, Neoptolemos JP (2001) Detailed tissue expression of bcl-2, bax, bak and bcl-x in the normal human pancreas and in chronic pancreatitis, ampullary and pancreatic ductal adenocarcinomas. *Pancreatology* **1:** 254–262

Farrell JJ, Elsaleh H, Garcia M, Lai R, Ammar A, Regine WF, Abrams R, Benson AB, Macdonald J, Cass CE, Dicker AF, Mackey JR (2009) Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. *Gastroenterology* **136**(1)**:** 187–195

Friess H, Lu Z, Graber HU, Zimmermann A, Adler G, Korc M, Schmid RM, Büchler MW (1998) bax, but not bcl-2, influences the prognosis of human pancreatic cancer. *Gut* **43:** 414–421

Fujimoto K, Hosotani R, Wada M, Lee JU, Koshiba T, Miyamoto Y, Tsuji S, Nakajima S, Doi R, Imamura M (1998) Expression of two angiogenic factors, vascular endothelial growth factor and platelet-derived endothelial cell growth factor in human pancreatic cancer, and its relationship to angiogenesis. *Eur J Cancer* **34:** 1439–1447

Fujioka S, Yoshida K, Yanagisawa S, Kawakami M, Aoki T, Yamazaki Y (2001) Angiogenesis in pancreatic carcinoma: thymidine phosphorylase expression in stromal cells and intratumoral microvessel density as independent predictors of overall and relapse-free survival. *Cancer* **92:** 1788–1797

Gansauge F, Gansauge S, Link KH, Beger HG (1999) p53 in relation to therapeutic outcome of locoregional chemotherapy in pancreatic cancer. *Ann N Y Acad Sci* **880:** 281–287

Gansauge F, Gansauge S, Schmidt E, Müller J, Beger HG (1998) Prognostic significance of molecular alterations in human pancreatic carcinoma – an immunohistological study. *Langenbecks Arch Surg* **383:** 152–155

Gazzaniga GM, Papadia FS, Dezzana M, Cappato S, Filauro M, Bandelloni R (2001) Role of p53 mutations on survival after pancreatoduodenectomy for ductal adenocarcinoma of the pancreatic head. *Hepatogastroenterology* **48:** 1743–1745

Gerdes B, Ramaswamy A, Ziegler A, Lang SA, Kersting M, Baumann R, Wild A, Moll R, Rothmund M, Bartsch DK (2002) p16INK4a is a prognostic marker in resected ductal pancreatic cancer: an analysis of p16INK4a, p53, MDM2, an Rb. *Ann Surg* **235:** 51–59

Hamacher R, Schmid RM, Saur D, Schneider G (2008) Apoptotic pathways in pancreatic ductal adenocarcinoma. *Mol Cancer* **7:** 64

Hashimoto K, Nio Y, Koike M, Itakura M, Yano S, Higami T, Maruyama R (2005) Expression of retinoblastoma and p53 pathway-related proteins in resectable invasive ductal carcinoma of the pancreas: potential cooperative effects on clinical outcome. *Anticancer Res* **25:** 1361–1368

Hayden JA, Côté P, Bombardier C (2006) Evaluation of the quality of prognosis studies in systematic reviews. *Ann Intern Med* **144:** 427–437

Hermanova M, Karasek P, Nenuti R, Kyr M, Tomasek J, Baltasova I, Dite P (2009) Clinicopathological correlations of cyclooxygenase-2, MDM2, and p53 expressions in surgically resectable pancreatic invasive ductal adenocarcinoma. *Pancreas* **38:** 565–571

Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* **21:** 1539–1558

Hu YX, Watanabe H, Ohtsubo K, Yamaguchi Y, Ha A, Motoo Y, Okai T, Sawabu N (1999) Bcl-2 expression related to altered p53 protein and its impact on the progression of human pancreatic carcinoma. *Br J Cancer* **80:** 1075–1079

Hu YX, Watanabe H, Ohtsubo K, Yamaguchi Y, Ha A, Okai T, Sawabu N (1997) Frequent loss of p16 expression and its correlation with clinicopathological parameters in pancreatic carcinoma. *Clin Cancer Res* **3:** 1473–1477

Hua Z, Zhang YC, Hu XM, Jia ZG (2003) Loss of DPC4 expression and its correlation with clinicopathological parameters in pancreatic carcinoma. *World J Gastroenterol* **9:** 2764–2767

Ikeda N, Adachi M, Taki T, Huang C, Hashida H, Takabayashi A, Sho M, Nakajima Y, Kanehiro H, Hisanaga M, Nakano H, Miyake M (1999) Prognostic significance of angiogenesis in human pancreatic cancer. *Br J Cancer* **79:** 1553–1563

Ikeda N, Nakajima Y, Sho M, Adachi M, Huang CL, Iki K, Kanehiro H, Hisanaga M, Nakano H, Miyake M (2001) The association of K-ras gene mutation and vascular endothelial growth factor gene expression in pancreatic carcinoma. *Cancer* **92:** 488–499

Itakura J, Ishiwata T, Friess H, Fujii H, Matsumoto Y, Büchler MW, Korc M (1997) Enhanced expression of vascular endothelial growth factor in human pancreatic cancer correlates with local disease progression. *Clin Cancer Res* **3:** 1309–1316

Jeong J, Park YN, Park JS, Yoon DS, Chi HS, Kim BR (2005) Clinical significance of p16 protein expression loss and aberrant p53 protein expression in pancreatic cancer. *Yonsei Med J* **46:** 519–525

Jinfeng M, Kimura W, Sakurai F, Moriya T, Mizutani M, Hirai I (2007) Prognostic role of angiogenesis and its correlations with thymidine phosphorylase and p53 expression in ductal adenocarcinoma of the pancreas. *Hepatogastroenterology* **54:** 1635–1640

Karademir S, Sökmen S, Terzi C, Sağol O, Ozer E, Astarcioğlu H, Coker A, Astarcioğlu I (2000) Tumor angiogenesis as a prognostic predictor in pancreatic cancer. *J Hepatobiliary Pancreat Surg* **7:** 489–495

Kawesha A, Ghaneh P, Andrén-Sandberg A, Ograed D, Skar R, Dawiskiba S, Evans JD, Campbell F, Lemoine N, Neoptolemos JP (2000) K-ras oncogene subtype mutations are associated with survival but not expression of p53, p16(INK4A), p21(WAF-1), cyclin D1, erbB-2 and erbB-3 in resected pancreatic ductal adenocarcinoma. *Int J Cancer* **89:** 469–474

Khorana AA, Hu YC, Ryan CK, Komorowski RA, Hostetter G, Ahrendt SA (2005) Vascular endothelial growth factor and DPC4 predict adjuvant therapy outcomes in resected pancreatic cancer. *J Gastrointest Surg* **9:** 903–911

Knoll MR, Rudnitzki D, Sturm J, Manegold BC, Post S, Jaeger TM (2001) Correlation of postoperative survival and angiogenic growth factors in pancreatic carcinoma. *Hepatogastroenterology* **48:** 1162–1165

Kurahara H, Takao S, Maemura K, Shinchi H, Natsugoe S, Aikou T (2004) Impact of vascular endothelial growth factor-C and -D expression in human pancreatic cancer: its relationship to lymph node metastasis. *Clin Cancer Res* **10:** 8413–8420

Kuwahara K, Sasaki T, Kuwada Y, Murakami M, Yamasaki S, Chayama K (2003) Expressions of angiogenic factors in pancreatic ductal carcinoma: a correlative study with clinicopathologic parameters and patient survival. *Pancreas* **26:** 344–349

Kyzas PA, Cunha IW, Ioannidis JP (2005a) Prognostic significance of vascular endothelial growth factor immunohistochemical expression in head and neck squamous cell carcinoma: a meta-analysis. *Clin Cancer Res* **11:** 1434–1440

Kyzas PA, Loizou KT, Ioannidis JP (2005b) Selective reporting biases in cancer prognostic factor studies. *J Natl Cancer Inst* **97:** 1043–1055

Lee EU, Cibull ML, O'Daniel-Pierce E, Strodel WE, Jennings CD (1995) Expression of p53 protein in pancreatic adenocarcinoma. Relationship to cigaret smoking. *Int J Pancreatol* **17:** 237–242

Li Y, Bhuiyan M, Vaitkevicius VK, Sarkar FH (1999) Structural alteration of p53 protein correlated to survival in patients with pancreatic adenocarcinoma. *Pancreas* **18:** 104–110

Lim YJ, Lee JK, Park CK, Song SY, Jang WY, Ha HY, Park DI, Lee KT, Paik SW, Yoo BC, Rhee JC (2004) Prognostic value of VEGF in human pancreatic ductal adenocarcinoma. *Korean J Intern Med* **19:** 10–14

Linder S, Blåsjö M, von Rosen A, Parrado C, Falkmer UG, Falkmer S (2001) Pattern of distribution and prognostic value of angiogenesis in pancreatic duct carcinoma: a semiquantitative immunohistochemical study of 45 patients. *Pancreas* **22:** 240–247

Linder S, Parrado C, Falkmer UG, Blåsjö M, Sundelin P, von Rosen A (1997) Prognostic significance of Ki-67 antigen and p53 protein expression in pancreatic duct carcinoma: a study of the monoclonal antibodies MIB-1 and DO-7 in formalin-fixed paraffin-embedded tumour material. *Br J Cancer* **76:** 54–59

Lundin J, Nordling S, von Boguslawsky K, Roberts PJ, Haglund C (1996) Prognostic value of immunohistochemical expression of p53 in patients with pancreatic cancer. *Oncology* **53:** 104–111

Magistrelli P, Coppola R, Tonini G, Vincenzi B, Santini D, Borzomati D, Vecchio F, Valeri S, Castri F, Antinori A, Nuzzo G, Caraglia M, Picciocchi A (2006) Apoptotic index or a combination of Bax/Bcl-2 expression correlate with survival after resection of pancreatic adenocarcinoma. *J Cell Biochem* **97:** 98–108

Mäkinen K, Hakala T, Lipponen P, Alhava E, Eskelinen M (1998) Clinical contribution of bcl-2, p53 and Ki-67 proteins in pancreatic ductal adenocarcinoma. *Anticancer Res* **18:** 615–658

Malats N, Bustos A, Nascimento CM, Fernandez F, Rivas M, Puente D, Kogevinas M, Real FX (2005) P53 as a prognostic marker for bladder cancer: a meta-analysis and review. *Lancet Oncol* **6:** 678–686

Manuyakorn A, Paulus R, Farrell J, Dawson NA, Tze S, Cheung-Lau G, Hines OJ, Reber H, Seligson DB, Horvath S, Kurdistani SK, Dawson DW (2010) Cellular histone modification patterns predict prognosis and

treatment response in resectable pancreatic adenocarcinoma: results from RTOG 9704. *J Clin Oncol* **28:** 1358–1365

Martin B, Paesmans M, Berghmans T, Branle F, Ghisdal L, Mascaux C, Meert AP, Steels E, Vallot F, Verdebout JM, Lafitte JJ, Sculier JP (2003) Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer* **89:** 55–64

McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2006) Reporting recommendations for tumor marker prognostic studies (REMARK). *Exp Oncol* **28:** 99–105

Naka T, Kobayashi M, Ashida K, Toyota N, Kaneko T, Kaibara N (1998) Aberrant p16INK4 expression related to clinical stage and prognosis in patients with pancreatic cancer. *Int J Oncol* **12:** 1111–1116

Nash AD, Baca M, Wright C, Scotney PD (2006) The biology of vascular endothelial growth factor-B (VEGF-B). *Pulm Pharmacol Ther* **19:** 61–69

Neoptolemos JP, Stocken DD, Friess H, Bassi C, Dunn JA, Hickey H, Beger H, Fernandez-Cruz L, Dervenis C, Lacaine F, Falconi M, Pederzoli P, Pap A, Spooner D, Kerr DJ, Büchler MW, European Study Group for Pancreatic Cancer (2004) A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med* **350:** 1200–1210

Niedergethmann M, Hildenbrand R, Wolf G, Verbeke CS, Richter A, Post S (2000) Angiogenesis and cathepsin expression are prognostic factors in pancreatic adenocarcinoma after curative resection. *Int J Pancreatol* **28:** 31–39

Niedergethmann M, Hildenbrand R, Wostbrock B, Hartel M, Sturm JW, Richter A, Post S (2002) High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas. *Pancreas* **25:** 122–129

Nio Y, Dong M, Uegaki K, Hirahara N, Minari Y, Sasaki S, Takamura M, Iguchi C, Tamura K (1998) p53 expression affects the efficacy of adjuvant chemotherapy after resection of invasive ductal carcinoma of the pancreas. *Anticancer Res* **18:** 3773–3779

Nio Y, Dong M, Uegaki K, Hirahara N, Minari Y, Sasaki S, Takamura M, Iguchi C, Tamura K (1999) Comparative significance of p53 and WAF/1-p21 expression on the efficacy of adjuvant chemotherapy for resectable invasive ductal carcinoma of the pancreas. *Pancreas* **18:** 117–126

Nio Y, Dong M, Iguchi C, Yamasawa K, Toga T, Itakura M, Tamura K (2001a) Expression of Bcl-2 and p53 protein in resectable invasive ductal carcinoma of the pancreas: effects on clinical outcome and efficacy of adjuvant chemotherapy. *J Surg Oncol* **76:** 188–196

Nio Y, Iguchi C, Yamasawa K, Sasaki S, Takamura M, Toga T, Dong M, Itakura M, Tamura K (2001b) Apoptosis and expression of Bcl-2 and Bax proteins in invasive ductal carcinoma of the pancreas. *Pancreas* **22:** 230–239

Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutberlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B, Riess H (2007) Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA* **297:** 267–277

Ohshio G, Suwa H, Imamura M (2002) Clinical implication of anti-p53 antibodies and p53-protein in pancreatic disease. *Int J Gastrointest Cancer* **31:** 129–135

Ohshio G, Suwa H, Imamura T, Yamaki K, Tanaka T, Hashimoto Y, Imamura M (1998) An immunohistochemical study of bcl-2 and p53 protein expression in pancreatic carcinomas. *Scand J Gastroenterol* **33:** 535–539

Ohtsubo K, Watanabe H, Yamaguchi Y, Hu YX, Motoo Y, Okai T, Sawabu N (2003) Abnormalities of tumor suppressor gene p16 in pancreatic carcinoma: immunohistochemical and genetic findings compared with clinicopathological parameters. *J Gastroenterol* **38:** 663–671

Parmar MK, Torri V, Stewart L (1998) Extracting summary statistics to perform meta-analyses of the published literature for survival end-points. *Stat Med* **17:** 2815–2834

Riall TS, Cameron JL, Lillemoe KD, Winter JM, Campbell KA, Hruban RH, Chang D, Yeo CJ (2006) Resected periampullary adenocarcinoma: 5-year survivors and their 6- to 10-year follow-up. *Surgery* **140:** 764–772

Sarela AI, Verbeke CS, Ramsdale J, Davies CL, Markham AF, Guillou PJ (2002) Expression of survivin, a novel inhibitor of apoptosis and cell cycle regulatory protein, in pancreatic adenocarcinoma. *Br J Cancer* **86:** 886–892

Sato Y, Nio Y, Song MM, Sumi S, Hirahara N, Minari Y, Tamura K (1997) p53 protein expression as prognostic factor in human pancreatic cancer. *Anticancer Res* **17:** 2779–2788

Schutte M, Hruban RH, Geradts J (1997) Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res* **57:** 3126–3130

Seo Y, Baba H, Fukuda T, Takashima M, Sugimachi K (2000) High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. *Cancer* **88:** 2239–2245

Sessa F, Bonato M, Bisoni D, Ranzani GN, Capella C (1998) Ki-ras and p53 gene mutations in pancreatic ductal carcinoma: a relationship with tumor phenotype and survival. *Eur J Histochem* **42:** 67–76

Sinicrope FA, Evans DB, Leach SD, Cleary KR, Fenoglio CJ, Lee JJ, Abbruzzese JL (1996) bcl-2 and p53 expression in resectable pancreatic adenocarcinomas: association with clinical outcome. *Clin Cancer Res* **2:** 2015–2022

Smeenk HG, Erdmann J, van Dekken H, van Marion R, Hop WC, Jeekel J, van Eijck CH (2007) Long-term survival after radical resection for pancreatic head and ampullary cancer: a potential role for the EGF-R. *Dig Surg* **24:** 38–45

Stipa F, Lucandri G, Limiti MR, Bartolucci P, Cavallini M, Di Carlo V, D'Amato A, Ribotta G, Stipa S (2002) Angiogenesis as a prognostic indicator in pancreatic ductal adenocarcinoma. *Anticancer Res* **22:** 445–449

Sun CY, Wang BL, Hu CQ, Peng RY, Gao YB, Gu QY, Wang DW (2002) Expression of the bcl-2 gene and its significance in human pancreatic carcinoma. *Hepatobiliary Pancreat Dis Int* **1:** 306–308

Takikita M, Altekruse S, Lynch CF, Goodman MT, Hermandez BY, Green M, Cozen W, Cockburn M, Siburg Saber M, Topor M, Zeruto C, Abedi-Ardekani B, Reichman ME, Hewitt SM (2009) Associations between selected biomarkers and prognosis in a population-based pancreatic cancer tissue microarray. *Cancer Res* **69:** 2950–2955

Tascilar M, Skinner HG, Rosty C, Sohn T, Wilentz RE, Offerhaus GJ, Adsay V, Abrams RA, Cameron JL, Kern SE, Yeo CJ, Hruban RH, Goggins M (2001) The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res* **7:** 4115–4121

Tang RF, Itakura J, Aikawa T, Matsuda K, Fujii H, Korc M, Matsumoto Y (2001) Overexpression of lymphangiogenic growth factor VEGF-C in human pancreatic cancer. *Pancreas* **22:** 285–292

Tang RF, Wang SX, Peng L, Wang SX, Zhang M, Li ZF, Zhang ZM, Xiao Y, Zhang FR (2006) Expression of vascular endothelial growth factors A and C in human pancreatic cancer. *World J Gastroenterol* **12:** 280–286

Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR (2007) Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* **8:** 16

Toga T, Nio Y, Hashimoto K, Higami T, Maruyama R (2004) The dissociated expression of protein and messenger RNA of DPC4 in human invasive ductal carcinoma of the pancreas and their implication for patient outcome. *Anticancer Res* **24:** 1173–1178

Tomazic A, Pegan V, Ferlan-Marolt K, Pleskovic A, Luzar B (2004) Cyclin D1 and bax influence the prognosis after pancreatoduodenectomy for periampullary adenocarcinoma. *Hepatogastroenterology* **51:** 1832–1837

Ueda S, Hatsuse K, Tsuda H, Ogata S, Kawarabayashi N, Takigawa T, Einama T, Morita D, Fukatsu K, Sugiura Y, Matsubara O, Mochizuki H (2006) Potential crosstalk between insulin-like growth factor receptor type 1 and epidermal growth factor receptor in progression and metastasis of pancreatic cancer. *Mod Pathol* **19:** 788–796

Ueda S, Ogata S, Tsuda H, Kawarabayashi N, Kimura M, Sugiura Y, Tamai S, Matsubara O, Hatsuse K, Mochizuki H (2004) The correlation between cytoplasmic overexpression of epidermal growth factor receptor and tumor aggressiveness: poor prognosis in patients with pancreatic ductal adenocarcinoma. *Pancreas* **29:** 1–8

Uegaki K, Nio Y, Inoue Y, Minari Y, Sato Y, Song MM, Dong M, Tamura K (1997) Clinicopathological significance of epidermal growth factor and its receptor in human pancreatic cancer. *Anticancer Res* **17:** 3841–3847

Virkajärvi N, Pääkkö P, Soini Y (1997) Association between p53 overexpression, cell proliferation, tumor necrosis and extent of apoptosis in operated pancreatic adenocarcinoma. *APMIS* **105:** 765–772

Westphal S, Kalthoff H (2003) Apoptosis: targets in pancreatic cancer. *Mol Cancer* **2:** 6

Weyrer K, Feichtinger H, Haun M, Weiss G, Ofrer D, Weger AR, Umlauft F, Grunewald K (1996) p53, Ki-ras, and DNA ploidy in human pancreatic ductal adenocarcinoma. *Lab Invest* **74:** 279–289

Wilentz RE, Iacobuzio-Donahue CA, Argani P (2000) Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. *Cancer Res* **60:** 2002–2006

Williamson PR, Smith CT, Hutton JL, Marson AG (2002) Aggregate data meta-analysis with time-to-event outcomes. *Stat Med* **21:** 3337–3351

Yamaguchi K, Chijiiwa K, Torato N, Kinoshita M, Tanaka M (2000) Ki-ras codon 12 point and P53 mutations: a molecular examination of the main tumor, liver, portal vein, peripheral arterial blood and para-aortic lymph node in pancreatic cancer. *Am J Gastroenterol* **95:** 1939–1945

Yamanaka Y, Friess H, Kobrin MS, Buchler M, Beger HG, Korc M (1993) Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. *Anticancer Res* **13:** 565–569

Yamasawa K, Nio Y, Dong M, Yamaguchi K, Itakura M (2002) Clinicopathological significance of abnormalities in Gadd45 expression and its relationship to p53 in human pancreatic cancer. *Clin Cancer Res* **8:** 2563–2569

Yamazaki Y, Morita T (2006) Molecular and functional diversity of vascular endothelial growth factors. *Mol Divers* **10:** 515–527

Yokoyama M, Yamanaka Y, Friess H, Buchler M, Korc M (1994) p53 expression in human pancreatic cancer correlates with enhanced biological aggressiveness. *Anticancer Res* **14:** 2477–2483

Yu G, Zhu MH, Zhu Z, Ni CR, Zheng JM, Li FM (2004) Expression of ATM protein and its relationship with p53 in pancreatic carcinoma with tissue array. *Pancreas* **28:** 421–426

Zhang SY, Ruggeri B, Agarwal P, Sorling AF, Obara T, Ura H, Namiki M, Klein-Szanto AJ (1994) Immunohistochemical analysis of p53 expression in human pancreatic carcinomas. *Arch Pathol Lab Med* **118:** 150–154

Zhang B, Zhao WH, Zhou WY, Yu WS, Yu JM, Li S (2007) Expression of vascular endothelial growth factors-C and -D correlate with evidence of lymphangiogenesis and angiogenesis in pancreatic adenocarcinoma. *Cancer Detect Prev* **31:** 436–442

Zhang L, Yuan SZ (2002) Expression of c-erbB-2 oncogene protein, epidermal growth factor receptor, and TGF-beta1 in human pancreatic ductal adenocarcinoma. *Hepatobiliary Pancreat Dis Int* **1:** 620–623